Prevention and treatment of infections in patients with haematological malignancies

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2008

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The publication of this thesis was financially supported by Amgen Oncology, Bayer Schering Pharma BV, Bristol-Myers Squibb BV, Celgene BV, Leo Pharma BV, Novartis Oncology, Ortho Biotech, a division of Janssen-Cilag BV, Roche Nederland BV, Shire International Licensing BV, Sanofi Aventis and Freesia Funding Leiden.

Cover design and layout by Jozien & Gert Jan Timmers

Printed by Buijten & Schipperheijn, Amsterdam, the Netherlands
ISBN/EAN: 978-90-9022950-8

VRIJE UNIVERSITEIT

Prevention and treatment of infections in patients with haematological malignancies

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad Doctor aan
de Vrije Universiteit Amsterdam,
op gezag van de rector magnificus
prof.dr. L.M. Bouter,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
van de faculteit der Geneeskunde
op vrijdag 23 mei 2008 om 10.45 uur
in het auditorium van de universiteit,
De Boelelaan 1105

door

Gerard Jan Timmers

geboren te Leiden

promotor: prof.dr. P.C. Huijgens

copromotor: dr. A.M. Simoons-Smit

Voor Erica, Jozien en Annemarieke,
in liefdevolle herinnering aan Myrthe

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Chapter 1

Background of the thesis, aims and outline

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In the Netherlands, approximately 73.000 new patients are diagnosed with cancer every year, with a yearly mortality of about 38.000 patients. Malignancies of the blood and bone marrow represent less than 10% of all cancers, which in the Netherlands accounts for 7000 patients diagnosed with a haematological malignant disease every year and a yearly mortality among these patients of about 3000 subjects. Over the past decades, the cure rate and prognosis of patients with haematological disease has improved substantially.[1] Above all, this may be attributed to advancements in therapy, since treatment options for patients with haematological malignancies have expanded and greatly improved. Unfortunately, the progress in treatment for haematological malignancies comes with the cost of serious impairment of host defence mechanisms. This occurs in a population of patients that is already running a serious risk of infections, due to the underlying disease, which resides by definition in the immune system itself.

Autologous stem cell transplantation is a valuable option for the treatment of patients with, for example, aggressive or relapsed lymphoma, acute leukemia and multiple myeloma.[2-6] These patients however, suffer from a temporary lack of neutrophil granulocytes until engraftment occurs and in addition, dose-intensified chemotherapeutic regimens may lead to damage of mucosal surfaces. Amongst others, both neutropenia and the occurrence of mucositis have been identified as important risk factors for the acquisition of serious opportunistic infections in these patients.[7-9]

Allogeneic stem cell transplantation has also proven to be a powerful tool in the treatment of a variety of haematological diseases.[10-13] However, success can not be achieved without the application of immunosuppressive drugs, first for the prevention of graft failure and later for the control of graft-versus-host disease. From these, it becomes evident that deficiencies in host defence mechanisms in allogeneic stem cell recipients are multiple, rendering these patients at a high risk of contracting serious viral, bacterial and fungal infections.[14-19]

Apart from the transplantation setting, in the past two decades immunotherapeutics have been successfully introduced in treatment schedules for haematological disease. Monoclonal antibodies including rituximab and alemtuzumab and drugs like the purine analogs such as fludarabin, have become widely used to date. These drugs however, directly interfere with B- and T-cell function, and their application has been paralelled by the emergence of infections by a variety of microorganisms, such as listeriosis, pneumocystosis, mycobacterial infections, and fungal and viral infections.[20-23]

Infectious complications seriously hamper the treatment of patients with haematological malignancies and are associated with severe morbidity and mortality. Hence, to date, it becomes evident that the survival of these patients heavily depends on the quality of supportive care. The search for ways to prevent and if necessary treat these infections has tried to keep pace with the improvement and intensification of treatment strategies. In view of the serious consequences of infections in patients treated for haematological disease, it is not surprising that newly marketed and promising antibacterial and antifungal drugs are readily introduced into daily clinical practice. Yet, sometimes important questions remain to be answered, with special interest to the population involved. Antibacterial or antifungal drugs may be adequately tested in healthy volunteers and be found safe and effective as treatment for short periods of use in the general population. However, when these drugs are applied in patients with haematological disease, quite often no data exist on safety, pharmacokinetic behaviour and their efficacy for prophylaxis or treatment in this specific population. Pharmacokinetic properties of antimicrobial agents, such as absorption, distribution and elimination may be significantly altered in patients with haematological malignancies, due to, for example, the underlying disease, low albumin state, the occurrence of graft-versus-host disease and mucosal damage.[24-26] Furthermore, when antibiotics are applied for antibacterial or antifungal prophylaxis, prolonged administration may lead to the emergence of resistant microorganisms.

In view of these considerations, this thesis aims to explore the safety and efficacy of a selection of new antimicrobials, for the prevention and treatment of bacterial and fungal infections in patients who are hospitalized for the treatment of a haematological malignancy. **Chapter 2** provides an overview from a clinical perspective of current insights and developments on antibacterial and antifungal prophylaxis and therapy in neutropenic patients. In **chapter 3** the pharmacokinetic properties of levofloxacin are explored, when used as antibacterial prophylaxis. Levofloxacin is a relatively new quinolone with enhanced activity against Gram-positive bacteria, and special attention is aimed at its effects on the microflora of the digestive tract. The findings in this study led to the design of a randomized clinical trial, comparing levofloxacin with ciprofloxacin-phenethicillin as antibacterial prophylaxis, the results of which are described in **chapter 4**. In **chapter 5** the use of amphotericicn B colloidal dispersion as antifungal prophylaxis is described, with an emphasis on toxicity of the drug. **Chapter 6** includes the results of a clinical trial that was designed to investigate the effects of cyclosporin A on single dose pharmacokinetics of itraconazole. The introduction of

cefpirome as empirical antibacterial treatment in neutropenic patients with fever, led to the design of an observational cohort study to evaluate its efficacy and safety, the results of which are described in **chapter 7**. In addition pharmacokinetic data were collected in a subset of patients to define the optimal dosing regimen for cefpirome. In **chapter 8,** the occurrence of a nosocomial outbreak of vancomycin-resistant *Enterococcus faecium* on our ward is described, including the results of a risk-factors analysis and measures that led to control of the epidemic. **Chapter 9** summarizes and discusses the main findings of the thesis.

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Chapter 2

General introduction

I Antibacterial prophylaxis

II Antifungal prophylaxis

III If prophylaxis fails, the approach to the patient with febrile neutropenia

IV Conclusion

Introduction

Over the past decades much progress has been made in the treatment and cure of patients with haematological malignancies. This, undoubtedly, can be attributed to improvement of intensive chemotherapy regimens and stem cell transplantation procedures, as well as to the application of novel immunomodulating agents such as purine analogs and monoclonal antibodies. These intensive treatment strategies, however, together with the underlying disease, lead to a substantial impairment of host immune response mechanisms, rendering these patients at a high risk of acquiring infections, associated with significant morbidity and mortality. In patients with acute leukemias, for example, more than 80% of cycles of intensive chemotherapy are complicated by fever or infections.[1,2] Profound neutropenia (neutrophil count < 0.1x 10^9 cells/L) is associated with a risk of developing bacteremia of about 20%.[3,4] The degree of neutropenia and its duration have been identified as principal risk factors associated with the occurrence of fever and serious infections in patients with neutropenia.[3,5,6] Other risk factors include the use of steroids and the application of indwelling catheters. In addition, infectious complications may be promoted by cytotoxic therapy-induced damage of mucosal surfaces, impaired peristaltic activity of the bowel and changes in the gut flora.[7-10]

Signs and symptoms of infection can be minimal in the patient with neutropenia. Fever may be the first and sometimes only manifestation of serious infection, and even the febrile response may be blunted if steroids or other immunosuppressive agents are used.[11,12] In these patients rapidly progressive infections may be life-threatening, and a prompt clinical evaluation to identify the cause of the fever is mandatory.[12-14] A physical examination, repeated daily, a chest X-ray, complete blood cell count and kidney and liver function tests should be performed, together with blood cultures drawn from a peripheral vein and through the central venous catheter.[15] Additional culture specimens should be taken from all clinically suspected sites. Following this systematic evaluation, the cause of fever can be microbiologically documented by means of positive cultures of blood or other normally sterile sites in approximately 20% of cases (microbiologically documented infection; MDI). In about 30% of cases only clinical signs of the cause of fever may be obtained (clinically documented infection; CDI). Nevertheless, despite a well performed clinical evaluation, about 50% of all febrile episodes will remain without a clear focus or causative microorganism. These cases represent the population of patients with 'fever of unknown origin' (FUO).

The epidemiology of microorganisms involved has changed markedly over the past three decades. In the 1960s and 1970s Gram-negative microorganisms predominated the pattern of

infection in the United States and Europe, including *Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella* species.[12] In contrast, Gram-positive cocci, including *Staphylococcus aureus*, coagulase-negative staphylococci and viridans group (VG) streptococci are now by far the most frequently isolated pathogens.[16] Presumably, this shift towards infections with more Gram-positive microorganisms may be due to the widespread application of prophylactic antibiotics such as the fluoroquinolones, the use of indwelling catheters and chemotherapeutic regimens that cause more severe mucosal damage.[7] In addition to the changing epidemiology of bacterial pathogens, the incidence of invasive fungal infections (IFI) has increased significantly among patients with neutropenia, especially among those who are receiving allogeneic stem cell transplantation.[17,18] The most frequently isolated fungal pathogen is *Candida albicans*. However, the last decade, infections by non-albicans species, such as *Candida glabrata, Candida krusei or Candida tropicalis* have become more frequent, presumably explained by the widespread use of fluconazole prophylaxis.[19,20] The incidence of mold infections has also changed substantially over the past 10 years. Not only is invasive aspergillosis (IA) encountered more frequently, but also infections caused by molds that exhibit resistance to conventional antifungal agents, such as *Fusarium* species and the Zygomycetes, have gained clinical importance.[21-23]

In view of the frequently erratic presentation of infections in patients with aggressive haematological malignancies, with sometimes only minimal signs and symptoms, and the severity of infection-related morbidity and mortality, it is not surprising that anti-infective strategies have focused on prevention and early treatment of these infections. Antibacterial and antifungal prophylaxis and early, or 'empirical' antibiotic therapy have gained a lot of interest in the international literature but obviously, have also led to much controversy. This chapter will focus on current insights and developments on these topics, from a clinical perspective.

I Antibacterial prophylaxis

Prophylaxis of bacterial and fungal infections was introduced when it became clear that 80% of the infecting pathogens were originating from the patient's endogenous flora, and that about half of them were acquired during the hospital stay.[24] Selective elimination of pathogenic Gram-negative aerobic microorganisms and of the fungal flora from the digestive tract, while maintaining the non-pathogenic anaerobic flora, was shown to reduce the number

of infections and fever in patients with leukaemia.[25] However, the combination of oral non-absorbable and absorbable antibiotics commonly used for this type of prophylaxis, including colistin, vancomycin, neomycin, amphotericin B and cotrimoxazole, led to nausea and diarrhoea. Consequently, poor compliance increased the risk of recolonization and infections with other opportunistic and resistant pathogens. Moreover, prophylaxis with cotrimoxazole was complicated by hypersensitivity reactions and prolonged neutropenia.[26-28] Nevertheless, cotrimoxazole was widely applied for this purpose and has been shown in meta-analyses to reduce the rate of bacterial infections in neutropenic patients significantly.[29-31] From the 1980s, fluoroquinolones became an attractive alternative for prophylaxis in neutropenic patients, because these drugs have a broad antimicrobial spectrum, are well absorbed, preserve the anaerobic flora of the digestive tract, and are generally well tolerated.[26,32-34] In placebo-controlled trials, fluoroquinolones were shown to reduce the incidence of Gram-negative bacteremia during neutropenia, but no significant effects could be demonstrated on the use of intravenous antibiotics or infection-related mortality.[35,36] Moreover, it became evident that the use of quinolone prophylaxis was hampered by an increasing incidence of infections with resistant Gram-negative microorganisms, such as *Escherichia coli*, but also of Gram-positive microorganisms, including coagulase-negative staphylococci and streptococci.[37-40] The latter resulted in clinical trials that investigated the addition of Gram-positive coverage to fluoroquinolone prophylaxis. A meta-analysis of nine of these studies showed that a reduction in bacteremia, fever, streptococcal and coagulase-negative staphylococcal infections could be achieved, but no improvement in clinically documented infections or infection-related mortality was found.[41] Moreover, Gram-positive prophylaxis significantly increased side-effects. More recently marketed quinolones, including levofloxacin, have more potent activity against Gram-positive microorganisms and may potentially overcome the problem of Gram-positive break-through infections. Two large multi-centre, randomized, double-blind, placebo-controlled trials, evaluating levofloxacin, were recently published. The SIGNIFICANT trial addressed the use of levofloxacin in patients receiving chemotherapy for solid tumours and lymphoma.[42] During the entire course of chemotherapy, the risk of fever was reduced approximately by one third, febrile episodes occurred in 10.8% of patients in the levofloxacin group versus 15.2% in the placebo group. The incidence of severe infection in the levofloxacin group was half that of the placebo group, but this was not a statistically significant difference and there was no difference in infection-related deaths between the two groups. The GIMEMA study looked at levofloxacin prophylaxis in a population of high-risk patients with acute leukaemia and high-dose

chemotherapy, and also reported significant improvements in all infection-related outcomes, but not in mortality.[43] A greater number of levofloxacin-resistant Gram-negative strains was found among patients receiving levofloxacin, but the presence of fluoroquinolone resistance did not seem to affect infection-related morbidity or mortality. Other reports indicate that the use of levofloxacin as antibacterial prophylaxis may be associated with the selection of VG streptococci that are resistant to quinolones with enhanced activity against Gram-positive organisms.[44-46] The occurrence of subsequent VG streptococcal bacteremia has been observed, which can be accompanied by serious complications, like adult respiratory distress syndrome (ARDS), shock and endocarditis. Mortality rates have been reported to range from 6% to 30%.[47]

The risk of selecting for resistant pathogens, together with the inability in clinical trials to demonstrate clinically significant reductions in overall mortality have resulted in international guidelines that do not advocate the routine application of antibacterial prophylaxis in all neutropenic patients.[27] However, more recently, large meta-analyses have become available, that address both the effects of quinolone prophylaxis on antimicrobial resistance and on mortality.[31,48] The first study assessed 56 trials for colonization with quinolone-resistant bacteria following prophylaxis.[48] Quinolone prophylaxis resulted in a (non-significant) higher rate of colonization with resistant bacteria, as compared with placebo or no treatment, but no increase in the rate of quinolone-resistant infections was found. When infections did develop, 1/3 of the causative pathogens were found to be resistant to the administered quinolone. The issue of mortality was addressed in another meta-analysis, that encompassed 95 trials, comparing antibiotic prophylaxis with placebo or no intervention or another antibiotic, in afebrile neutropenic patients.[31] Prophylaxis significantly reduced total mortality by 33% (CI_{95}, 19% to 45%) and infection-related mortality by 42% (CI_{95}, 26% to 55%). Fluoroquinolone prophylaxis reduced the risk of death by 48% (CI_{95}, 33% to 65%) and of infection-related death by 62% (CI_{95}, 31% to 79%), while the risk for developing resistance and of experiencing adverse effects was not statistically significant increased. The authors conclude that the reduction of mortality probably outweighs detriments, such as adverse effects and development of resistance and that prophylaxis, preferably with a fluoroquinolone, should be considered for use in neutropenic patients. These recommendations are now corroborated by others and it is concluded by several authors that future studies, rather than trying to prove its efficacy, should focus on the identification of patients who benefit the most from antibacterial prophylaxis during neutropenia.[29,30]

II Antifungal prophylaxis

In haemato-oncological patients, the risk of acquiring or dying from an invasive fungal infection (IFI) has increased substantially over the past decades. In particular, the advent of allogeneic stem cell transplantation techniques has increased the risk of IFI, due to the possible events of prolonged neutropenia, graft failure, graft-versus-host disease (GVHD) and the use of corticosteroids and other immunosuppressives.[23,49,50] Furthermore, the risk of IFI may vary with the underlying disease, for example, AML patients are more likely to acquire aspergillosis.[23,51,52] If acquired, IFI poses a serious threat especially to the patient with neutropenia. That is because mortality rates from these infections are high, and vary between 20 and 40% for invasive *Candida* infections and between 50 and 90% for invasive aspergillosis.[21,53,54] In addition, IFI remains difficult to diagnose, as conventional clinical, radiological and microbiological techniques are insensitive, non-specific and often time consuming.[55,56] Despite the improvement of antifungal strategies and the availability of more potent antifungal antibiotics, the treatment of IFI remains difficult. These conditions justifiably supported the use of antifungal prophylaxis in neutropenic patients and, as a result, the prevention of IFI has gained a lot of scientific interest. However, despite the existence of more than 50 randomized clinical trials and several meta-analyses, still no consensus has been reached regarding its efficacy.[57-62]

Fluconazole

Fluconazole is the most extensively studied triazole. Two placebo-controlled studies, involving allogeneic transplant recipients, demonstrated that primary prophylaxis with fluconazole at a daily dose of 400 mg reduced the incidence of invasive fungal infections and attributable mortality.[63,64] In a longitudinal follow-up study, prolonged administration of fluconazole, for up to 75 days after the transplantation procedure, resulted in a persistent protection against infections with *Candida*, a lower incidence of intestinal GVHD and improved survival.[65] In contrast, the efficacy of fluconazole 400 mg/d in patients receiving chemotherapy for acute leukaemia or other haematological malignancies, has not been proven yet. In 2 placebo-controlled trials fluconazole 400 mg/d was compared with placebo for the prevention of fungal infections in patients undergoing chemotherapy for acute leukaemia or other haematological malignancies.[66,67] Fluconazole prevented colonization and superficial infections by *Candida* spp. other than *Candida krusei*, but no significant effects were found on the rate of proven IFI or mortality. Lower doses of fluconazole, from 50- to 200-mg/d have

been compared with other antifungal agents in clinical trials, with variable results, but the efficacy of these dosing regimens has not been proven in placebo-controlled studies.[68-72]
Fluconazole has a favourable safety profile and patient compliance is good. However, the drug is ineffective against *Aspergillus* spp. and promotes the emergence of fluconazole-resistant or less-susceptible organisms, such as *Candida krusei* and *Candida glabrata.* In one report a 40% colonization rate and a seven-fold increase in *Candida krusei* infections in patients on fluconazole prophylaxis was found.[73] In several large studies breakthrough infections with these pathogens have been documented.[63,66,74]

Itraconazole

Itraconazole is an azole agent, with activity against many opportunistic fungi that are resistant to fluconazole, including *Aspergillus* and some *Candida* species. Itraconazole is now available as an oral solution with hydroxypropyl-cyclodextrin, as well as an intravenous formulation. These formulations have substantially increased the bio-availability of itraconazole, since the absorption of the earlier capsule formulation was unpredictable.[68,75,76] However, poor tolerability of the oral solution, due to bad taste and gastro-intestinal side effects have limited its clinical usefulness, and drop-out rates in some clinical trials have been substantial.[49,69,77,78] Another potential drawback to the use of itraconazole is its interaction with a variety of other drugs, due to the metabolism by the cytochrome P450-3A4 (CYP3A4) enzyme system.[79,80] The disposition of itraconazole may be enhanced by several drugs, including anticonvulsives such as phenytoin and carbamazepin and by some tuberculostatics. Itraconazole itself may inhibit the metabolism of other drugs, including oral anticoagulants and, importantly, of cyclosporin A.[81] In a double-blind, placebo-controlled trial, oral itraconazole suspension was compared with placebo for the prevention of fungal infections in neutropenic patients with haematological malignancies.[77] In the itraconazole group death due to candidemia was significantly reduced. Less infections with *Aspergillus* were documented among itraconazole recipients, though this was not a statistically significant difference. No effects were documented on mortality. In randomized, comparative trials with fluconazole in allogeneic transplant recipients, itraconazole reduced proven IFI more effectively. However, improvement of IFI-related mortality was not documented.[49,78,82] Other, recently published trials, involving patients with haematological malignancies outside the allogeneic transplantation setting, failed to show an advantage of either fluconazole or itraconazole prophylaxis in terms of incidence of IFI and IFI-related mortality.[83,84]

Amphotericin B

Amphotericin B (AmB) has the broadest spectrum of antifungal activity, as compared with other antifungal drugs evaluated for prophylactic purposes in neutropenic patients. Its application as an oral suspension may reduce yeast colonization, but there is no evidence that oral administration can prevent IFI.[60,72,85] Intravenous administration of low doses of AmB (0.1-0.2 mg/kg) for prophylaxis of fungal infections in neutropenic patients has been compared with placebo in two randomized clinical trials and with fluconazole (200-400 mg/day orally or intravenously) in another two studies.[86-89] In comparison with placebo, amphotericin B was found to reduce yeast colonization.[86,87] A lower number of IFI was found in the treatment arm, but this was not a statistically significant difference. In addition, both studies documented a better survival in amphotericin B recipients, however, this could not be attributed to the prevention of IFI. In comparison with fluconazole, low dose amphotericin B was equally efficient in the prevention of IFI, but fluconazole was less toxic.[88,89] No survival benefits of either study drug were documented.[88,89] The introduction of lipid based formulations of amphotericin B made it possible to administer relatively higher doses with, in potential, less toxicity as compared with the conventional drug. In two randomized, placebo controlled, clinical trials, the administration of liposomal amphotericin B (doses; resp. 2mg/kg three times weekly and 1mg/kg daily) resulted in a reduction of fungal colonization, but not in a lower number of proven IFI.[90,91] In a recent, open label, comparative trial a reduction in the number of IFI was documented when liposomal AmB was compared with no treatment, but no statistically significant differences in mortality were found.[92] The results of these trials do not seem to offer sufficient support for the application of low-dose liposomal amphotericin B for antifungal prophylaxis in neutropenic patients.[93] Finally, AmB can be aerosolized and used as inhalation therapy for the prevention of *Aspergillus* infections.[94,95] However, in a prospective, randomized, multicenter trial no benefit from prophylactic aerosolized AmB could be demonstrated.[96]

Other antifungal agents used for prophylaxis

New azole agents, including voriconazole, ravuconazole and posaconazole and the echinocandins like micafungin and caspofungin may be (and sometimes have been readily) introduced into daily clinical practice as antifungal prophylaxis in patients with neutropenia. However, only a few of these drugs have been subject to properly designed randomized clinical trials. In a randomized clinical trial, micafungin (50 mg/d) was more efficient in the prevention of suspected, probable and proven IFI than fluconazole (400mg/d).[97] More patients

in the fluconazole arm had breakthrough infections with *Aspergillus* (7 versus 1), however, this difference was not statistically significant. No differences were found in attributable mortality. In another randomized study caspofungin was compared with intravenous itraconazole for antifungal prophylaxis in patients with acute leukaemia or myelodysplastic syndrome. No differences were found between the two drugs in terms of prevention of IFI and toxicity.[98] Recently, two controlled trials were reported, evaluating the use of posaconazole as antifungal prophylaxis.[50,99] In one study, patients with acute leukaemia or myelodysplastic syndrome received prophylaxis with either posaconazole or fluconazole or itraconazole. The incidence of proven and probable IFI was significantly lower among posaconazole recipients, as compared with patients receiving fluconazole or itraconazole.[99] In addition, invasive aspergillosis occurred significantly less in the posaconazole group and mortality from any cause was significantly lower in the posaconazole group than in the fluconazole or itraconazole group. The other study involved patients who developed GVHD after allogeneic stem cell transplantation.[50] Posaconazole as compared with fluconazole, appeared to be as effective as fluconazole in the prevention of IFI, but proven or probable invasive aspergillosis occurred significantly less in the posaconazole group than in the fluconazole group. The number of deaths from IFI was lower in the posaconazole group.

In summary, antifungal prophylaxis has shown to be effective in reducing breakthrough fungal infections in randomized trials, but the reduction of attributable mortality rates is less well confirmed. Nevertheless, single trials may not achieve adequate statistical power to detect statistically significant differences. Indeed, several meta-analyses have confirmed the reduction of IFI by antifungal prophylaxis as found or suggested in single trials and in addition, do document a significant reduction of IFI-related mortality.[58,59,61] In a review of the published meta-analyses, the authors conclude that antifungal prophylaxis does have such an impact on the incidence of IFI and on its mortality, that its application is the currently best available choice for high-risk patients.[54] In addition, they state that direct comparisons with fluconazole have demonstrated superiority of itraconazole, as can be concluded from 2 trials in allogeneic stem cell recipients.[49,78] They propose the use of antifungal prophylaxis with itraconazole as a standard procedure for these patients. The results of recent clinical trials on posaconazole documented promising results for this agent as antifungal prophylaxis during neutropenia.[50,99]

III If prophylaxis fails; the approach to the patient with febrile neutropenia

Empirical antibiotic therapy in patients with fever and neutropenia

Despite the application of antibacterial and antifungal prophylaxis, more than half of all neutropenic patients, and in some patients with acute leukemias even more than 80%, will ultimately develop fever during their scheduled treatment.[1,2] Since the early 1970s, it has been recognized that rapid intervention in these patients is mandatory, to prevent detrimental outcome. For example, mortality in neutropenic patients with Gram-negative bacteraemia can be as high as 40%, if untreated.[100,101] Prompt administration of broad-spectrum antibiotics before microbiological confirmation of infection, has been associated with greatly improved outcome for patients with neutropenia and fever.[2,6,12,34,102] In 8 therapeutic trials of the International Antimicrobial Therapy Group of the European Organization for Research and Treatment of Cancer (EORTC IATG) the 30-day mortality rate in patients with Gram-negative and Gram-positive bacteraemia is now as low as 10% and 6% respectively, which is a dramatic improvement as compared with 1978, when more than 20% of the patients with Gram-negative sepsis and about 15% of those with Gram-positive bacteraemia died.[103] As a result, this approach of 'empirical' administration of antibiotics has become common practice.

Choice of antibiotic in the empirical treatment regimen

For years, the combination of a beta-lactam and an aminoglycoside antibiotic has been considered the best therapeutic approach for the empirical treatment of fever in patients with neutropenia.[34,104,105] The advantages of this regimen included its wide spectrum of action and its potential synergistic activity against Gram-negative rods. In theory, combination therapy may reduce the emergence of resistant strains. Disadvantages of the beta-lactam aminogycoside combination included its toxicity, especially its effect on kidney function, a poor activity against staphylococci and streptococci, and possible development of resistance in Gram-negative microorganisms. Recently, several comparative trials have shown that monotherapy with broad-spectrum antibiotics with antipseudomonas activity, including third and fourth generation cephalosporins and the carbapenems, can be as effective as combination therapy for the treatment of fever in neutropenic patients, with considerable less toxicity.[106-116] Current guidelines support the use of cefepime, ceftazidime, imipenem-cilastatin or meropenem, as single beta-lactam antibiotics for this purpose.[117] In a recently published systematic review and meta-analysis of 33 randomized controlled trials, ceftazidime, piperacillin/tazobactam, imipenem/cilastatin and meropenem all appeared to be equally

effective as empirical therapy in patients with fever and neutropenia.[117] No differences were found between these agents in all-cause and infection-related mortality. The exception was cefepime, which was associated with a higher mortality rate, probably due to less efficacy. Moreover, the carbapenems were associated with fewer treatment modifications than ceftazidime, but adverse events, including pseudomembranous colitis occurred more frequently and mortality was similar.

Evaluation of response, modification and duration of empirical therapy

After the introduction of empirical antibiotic treatment, a favourable response, defined by the resolution of fever without treatment modification, will be achieved in approximately 50-60% of patients.[118-120] The expected time to defervescence for patients with high-risk neutropenia, treated with appropriate empirical antibacterial regimens will be in the order of 5 days.[6,121] Therefore, it would be reasonable not to change the initial regimen for the first 3-5 days, even if the patient remains febrile, but otherwise is stable clinically.[27] Clinical events that do justify treatment modification include progression of infection-related signs and symptoms or worsening of vital signs, regimen-related toxicity and identification of microorganisms resistant to the initial regimen. The duration of administration of empirical antibiotic after defervescence is a difficult issue.[14] If no infection is identified after 3 days of treatment, if the neutrophil count is >0.5 x 10^9/L and if the patient is afebrile for >48 h, antibiotic therapy may be stopped.[27] If the patient becomes afebrile but remains neutropenic, the proper antibiotic strategy is less well defined. In persistently neutropenic patients, who initially responded to empirical antibacterial therapy, a 41% rate of recrudescence has been described, unless the antibacterial regimen was continued until neutrophil recovery.[122] However, this approach may increase drug toxicity and the rate of superinfections and it appears reasonable for neutropenic patients who are clinically stable and who have no remaining signs or symptoms of infection, to have their use of systemic antibiotics stopped after 5–7 afebrile days.[14,27]

If fever persists after 3-5 days of empirical antibiotic therapy, without clinical or microbiological documentation of an infection, there are several strategies that can be followed. (1) continue treatment with the initial antibiotics, (2) change or add antibiotics, (3) or add an antifungal drug to the regimen, with or without changing the antibiotics.[27] In the following section we will briefly discuss the value of the empirical addition of a glycopeptide, (in general vancomycin) to the antibiotic regimen, and address the addition of antifungal therapy.

Addition of vancomycin to the empirical regimen

Until recently, the increasing rate of infections with Gram-positive microorganisms such as VG group streptococci, coagulase-negative staphylococci, enterococci and *Corynebacterium* species in patients with neutropenia frequently led to the incorporation of vancomycin into the initial empirical antibiotic regimen. This strategy, however, has not been proven to be of any benefit, as shown in randomized clinical trials evaluating the addition of vancomycin for this purpose.[123-126] Furthermore, the emergence of vancomycin-resistant enterococci (VRE) has become of great concern. Outbreaks of VRE have been described, involving haematology and oncology units, with important consequences for daily patient care on the ward.[127-130] Moreover, blood-stream infections with VRE have been associated with serious morbidity and mortality.[131] Therefore, official guidelines recommend to limit the use of vancomycin to specific indications and discourage its routine use in empirical antibiotic strategies in febrile neutropenia.[27,132]

Empirical antifungal therapy

About 5-10% of all febrile episodes in neutropenic patients will ultimately be proven to be caused by fungal infections.[17,18,133] In autopsy studies in patients with haematological malignancies, IFI was found in at least 25-30% of autopsies, of which 75% were not diagnosed ante mortem.[134,135] So, these infections are potentially fatal and remain difficult to diagnose in an early phase. Therefore, the empirical administration of antifungal agents in patients who remain febrile after a certain period of antibacterial treatment appears to be a tenable stategy. However, its scientific support is rather feeble and comes from two randomized prospective trials, performed in the 1980's. These trials suggested that up to one-third of febrile neutropenic patients who did not respond to a 1-week course of antibiotic therapy might have IFI and that the empirical use of amphotericin B (AmB) was associated with a trend towards a reduction of these infections.[136,137] Later, both studies were criticized to be underpowered, and neither showed a statistically significant improvement in mortality. Nevertheless, to date, most clinicians believe that patients who remain febrile and profoundly neutropenic for more than 5 days, despite the administration of broad-spectrum antibiotics, should be offered antifungal therapy, a strategy that is also recommended in official guidelines.[27]

Recently developed antifungal agents have been compared with AmB in phase III studies, as for safety and efficacy in neutropenic patients with persistent fever. In a randomized, double-blind, multicenter trial in neutropenic patients with persisting fever, liposomal AmB and

conventional AmB demonstrated similar effectiveness, although breakthrough IFI occurred more frequently with AmB.[138] Significantly fewer side effects were documented in liposomal AmB recipients, including nephrotoxicity and infusion related adverse events. In another comparative study, itraconazole and AmB had at least equivalent efficacy as empirical antifungal therapy in neutropenic patients, but itraconazole was associated with less toxicity.[139] In another study, voriconazole was not proven to be non-inferior to liposomal AmB, but evaluation of the composite score for success indicated that the two treatments were similar. Moreover, voriconazole was superior in reducing documented breakthrough IFI, infusion-related toxicity, and nephrotoxicity.[17] Caspofungin has been compared with liposomal AmB as well.[18] Similar effectiveness was demonstrated with respect to defervescence and breakthrough IFI, but caspofungin was associated with fewer side effects. Given the complexity and methodological variations in the design of modern antifungal trials, there are no generally accepted recommendations as to which antifungal agent should be used in febrile neutropenic patients. It is to the discretion of the physician to make individualized decisions regarding the most optimal treatment, taking into account the locally perceived risk of IFI, types of fungal species isolated and the availability of diagnostic procedures for the early documentation of IFI.

Pre-emptive antifungal therapy

Notwithstanding the serious consequences of fungal infections, most neutropenic patients will not develop IFI. Therefore, empirical antifungal therapy is expected to benefit only a minority of patients with neutropenia, at the cost of toxicity and expensive medication used redundantly by others. New diagnostic procedures may overcome the difficulty in making a timely diagnosis in case of IFI. These methods may be used to withheld empirical antifungal therapy, until an IFI is suspected or proven. At that time, so called pre-emptive antifungal treatment can be instituted. Serum *Aspergillus* galactomannan and beta-glucan assays have been accepted as an adjunct in diagnostic strategies and guidelines, currently in preparation by the Mycosis Study Group of the EORTC.[140] In a meta-analysis, the galactomannan essay had a sensitivity of 70% and a specificity of 89% for proven aspergillosis, with a high negative predictive value of 95-99% in populations with a prevalence of invasive aspergillosis of 5-10%.[56] Detection of beta-glucan appeared to be highly sensitive and specific for the detection of IFI in patients with AML and myelodysplastic syndrome, with a negative predictive value of 100%.[141] Data on the application of the beta-glucan essay in stem cell transplantation recipients are limited.[142] *Aspergillus* PCR has been described as a promising, sensitive and

early indicator of aspergillosis.[143-145] However, this technique is time consuming, requires appropriate laboratory facilities and still lacks standardization. Chest CT-scans in combination with serial serum galactomannan samples have been used to detect early aspergillosis in high-risk neutropenic patients.[146] This strategy successfully identified cases with aspergillosis and was able to reduce the use of empirical antifungal therapy. Obviously, a drawback to this strategy is its lacks of ability to identify infections with other fungi than *Aspergillus*.

Treatment of proven fungal infections

In case of a proven IFI, the choice of antifungal therapy can be targeted to the fungal species identified, and may be based on microbiological resistance patterns. About 50-60% of invasive *Candida* infections are caused by *Candida albicans*.[133] Fluconazole, at a dose of 400-800 mg/d, remains an effective therapy in patients that have not received this agent for prophylactic purposes.[147] However, fluconazole is not effective when non-albicans species are involved, including *Candida krusei*, that is resistant to fluconazole and *Candida glabrata*, that has a dose-dependent sensitivity. In general, these patients are treated with AmB or its related compounds. Various trials have been performed, mainly in non-neutropenic patients, to compare new antifungal agents with fluconazole or conventional AmB for the treatment of invasive *Candida* infections. Non-inferiority has been proven for caspofungin as compared with AmB, for voriconazole as compared with a combination of AmB and fluconazole, and for anidulafungin as compared with fluconazole.[148-150] Drug related toxicities were lower in the study arms with non-AmB recipients.

The treatment of proven *Aspergillus* infections remains a challenge, the attributable mortality rate may be as high as 90% in certain populations of patients with neutropenia.[21,133] Provided that treatment is initiated without delay, response rates of 30-60% may be achieved.[133] For years, AmB has been the gold standard for treatment of patients with proven infections with *Aspergillus*. However, a large randomized study, published in 2002, showed that initial therapy with voriconazole was associated with a significantly higher percentage of successful outcome, improved survival and fewer side effects as compared with AmB.[151] A recent, non-comparative multicenter study, confirmed the efficacy of voriconazole in the treatment of invasive aspergillosis in patients with haematological malignancies and allogeneic stem cell transplant recipients.[152] To date, voriconazole is generally considered to be first-choice therapy in patients with proven aspergillosis.[133] The efficacy and safety of posaconazole for the treatment of patients with proven aspergillosis was investigated in an open-label

multicenter study, using historical controls.[153] The overall response rate among posoconazole recipients was higher than among controls, and the authors conclude that posaconazole may be valuable as salvage therapy in patients with aspergillosis who are refractory to previous antifungal agents.[153] In another non-comparative trial, caspofungin was administered to 90 patients with invasive aspergillosis, who were refractory to or intolerant of other antifungal therapy.[154] A favourable response to caspofungin therapy was observed in 45% of patients, demonstrating the usefulness of caspofungin in the salvage treatment of aspergillosis as well. As with empirical antifungal therapy, there are no clear guidelines to the optimal treatment of invasive aspergillosis. Treatment choices are still to be made by the treating physician on the basis of a few clinical trials available.

IV Conclusions

Although there is now growing evidence that both prophylactic and empirical administration of antibacterial and antifungal antibiotics may be of benefit in patients with severe neutropenia, the reverse side of the medal displays the concerning emergence of resistant pathogens. Many reports on new prophylactic or therapeutic strategies have been followed by the documentation of emerging resistant microorganisms or nosocomial outbreaks on haematology or oncology wards.[19,38,129-131,155-157] Thus, not only the well-being of the individual patient is at risk, but also of the population at a large. Moreover, the emergence of resistant microorganisms may have considerable effects on daily care and management of haemato-oncolgy units and may substantially increase work load for medical staff and health-care costs.

Although these concerns argue against the widespread and unlimited use of antibiotics in patients with neutropenia, a balanced appraisal is needed. First, it is noteworthy that the emergence of resistant strains not necessarily leads to subsequent infection with the microorganism involved. For example, in large meta-analyses, comparing quinolones with placebo or no treatment for the prevention of bacterial infections during neutropenia, no differences were found between patients receiving prophylaxis or placebo in the number of infections caused by pathogens resistant to quinolones.[29,31,48] Second, the reduction in mortality and infection rates, as demonstrated in these reviews, appears to outweigh the detriments of emerging resistant microorganisms. Third, several observational studies that examined the effects on outcome of patients with neutropenia when the practice of antibiotic prophylaxis was interrupted or stopped, documented more episodes with fever or bacteremia

and even increased mortality during the periods when prophylaxis was not given.[156,158-161] Fourth, efforts are made to distinguish categories of risk levels for infectious complications among patients with neutropenia.[162-164] In selected groups of low-risk patients the antibiotic therapy may be simplified or even discontinued. Also in the area of empirical antifungal therapy the issue is no longer to chose the 'best' agent, but rather to identify the population of patients that is likely to benefit the most from a given agent.[52,165,166] The advent of diagnostic techniques may promote the incorporation of preemptive antibiotic strategies, which will reduce the empirical overtreatment of patients with persisting fever during the neutropenic episode.

So, rather than refraining from the routine use of antibiotics in patients with neutropenia, the clinician faces a challenge to chose the right antibiotic regimen for the right population of patients. Not only data from clinical trials should guide these decisions. Other factors, that are at least as important, include local bacteriological and epidemiological data, with an emphasis on resistance patterns of predominantly isolated microorganisms, as well as the utility of an antibiotic in daily practice, its user-friendliness to patients and nursing staff, its toxicity and costs.

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Chapter 3

Pharmacokinetics and effects on bowel and throat microflora of oral levofloxacin as antibacterial prophylaxis in neutropenic patients with haematological malignancies

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Bone Marrow Transplantation 2004;33:847-853.

Abstract

Gram-positive breakthrough infections pose a major drawback to the use of quinolones for antibacterial prophylaxis in neutropenic patients. Levofloxacin offers the advantage of an augmented Gram-positive spectrum and may potentially overcome this problem. In an open-label, clinical pilot study, we investigated the effects on throat and bowel microflora and pharmacokinetics of a once-daily oral dose of 500 mg levofloxacin, during neutropenia in 20 patients with haematological malignancies. Gram-negative bowel flora and *Staphylococcus aureus* were successfully eradicated. No Gram-negative infections occurred. Minimal inhibitory concentration values for viridans group (VG) streptococci tended to increase, in four patients over 8 mg/l, indicating resistance to levofloxacin. Four patients developed blood-stream infections with levofloxacin-resistant Gram-positive cocci. No significant changes in numbers of anaerobic microorganisms were observed. Pharmacokinetic parameters of levofloxacin, including the maximum serum concentration (C_{max}), time to C_{max} (T_{max}), area under the concentration-time curve (AUC), volume of distribution at steady state (V_{ss}/F) and clearance (CL/F) were not statistically different at first dose and during neutropenia. In conclusion, levofloxacin eradicates Gram-negative microorganisms and *S. aureus* and spares the anaerobic flora. Its pharmacokinetic profile is unaltered during neutropenia. However, prolonged administration of levofloxacin as antibacterial prophylaxis may be hampered by the emergence of levofloxacin-resistant VG streptococci.

Introduction

Quinolones are widely used for the prevention of bacterial infections during neutropenia in patients with haemato-oncological diseases. The quinolones are attractive for this purpose because they are active against a broad range of bacteria and are well tolerated. Moreover, quinolones have been shown to reduce the incidence of Gram-negative infections and fever in neutropenic patients significantly, although a reduction of mortality does not seem to occur.[1] Of concern is the increased incidence of Gram-positive infections, which has been noted even in the first reports on the use of quinolones for the prevention of Gram-negative bacteraemia.[2,3] Especially, viridans group (VG) streptococci and coagulase-negative staphylococci have emerged as important pathogens in patients receiving quinolone prophylaxis.[2,4,5] To solve this problem, it has been attempted to augment the Gram-positive activity of the prophylactic regimen by the addition of a second antimicrobial agent, such as roxitromycin, rifampicin or penicillin. This approach indeed has been reported to result in significantly lower rates of Gram-positive infections.[5-10]

Recently, quinolone agents with enhanced Gram-positive activity have become available. A new fluoroquinolone, levofloxacin, shows excellent in vitro activity against many Gram-positive bacteria, including streptococci, enterococci and staphylococci, yet the drug retains the potent Gram-negative activity of earlier quinolones.[11,12] The pharmacokinetic profile of the drug is compatible with a once-daily dosing regimen. Considering these properties, levofloxacin seems an appealing agent for antimicrobial prophylaxis in neutropenic patients, and the drug has been readily applied as such by some centres. However, the consequences of this approach, from a microbiological point of view, remain largely unresolved. Issues like the development of antimicrobial resistance during prolonged administration of levofloxacin and its effect on microbiological flora have not been subject to prospective or systematic evaluation. Moreover, pharmacokinetics of levofloxacin have been studied in healthy volunteers only[13,14] and pharmacokinetic parameters such as absorption and bioavailability may be significantly altered in neutropenic patients, due to the underlying disease, chemotherapeutic regimen and the occurrence of mucosal damage.[15,16]

We therefore conducted this open-label, nonrandomized clinical pilot study to evaluate the pharmacokinetic profile of oral levofloxacin and to study its effect on throat and bowel microflora in neutropenic patients with haematological malignancies.

Patients and methods

Patients

Patients aged 18-75 years, hospitalized at the Haematology Department of the VU University Medical Centre and scheduled to receive high-dose combination chemotherapy, with or without autologous haematopoietic stem-cell rescue, were eligible for this study. An anticipated granulocytopenic period (granulocytes $<0.5 \times 10^9$/l) of at least 10 days was required.

Patients were excluded if they had a history of allergy to quinolones or if they had infection requiring treatment at entry. Treatment with any antimicrobial or antifungal drug within 2 weeks prior to enrolment was also a reason for exclusion, as was the use of aluminium or magnesium containing antacid drugs. Patients with hepatic or renal impairment, respectively, defined as elevation of any liver function test greater than three times the upper limit of normal or an estimated creatinine clearance of less than 15 ml/min, were not included. The aim of this study was to enroll 20 subjects. Patients who had to discontinue the study or were withdrawn for reasons not related to the study drug were to be replaced. The protocol was approved by the institutional scientific and ethical committees and all participating patients provided written informed consent.

Study drug and anti-infective measures

All patients received one 500 mg tablet of levofloxacin, once daily at 10.00 a.m. Treatment was started on the first day of chemotherapy and continued until recovery of neutrophils, defined as an absolute neutrophil count (ANC) $>0.5 \times 10^9$/l. In addition, for prevention purposes patients received fluconazole 50 mg once daily and nasal amphotericin B, 2 mg three times a day. Daily clinical assessments were performed, including documentation of signs and symptoms of infection and registration of compliance and tolerance to the study medication. If clinical signs and symptoms of infection occurred, or if axillary temperature increased above 38.5°C, patients were evaluated and started on empirical antibiotic treatment with imipenem-cilastatin 500 mg four times daily i.v. Prophylaxis with levofloxacin was to be continued. If no defervescence occurred within 4 × 24 h, amphotericin B 0.7 mg/kg i.v. was added to the antimicrobial regimen.

Microbiological methods

Throat swabs and faecal samples were collected before the first dose and afterwards twice weekly, on Tuesday and Friday. If the patient was unable to produce a faecal sample, a rectal-swab specimen was taken. Throat and rectal culture samples were obtained with soluble calcium-alginate swabs, stored in 10 ml transport medium. Subsequently, 0.1 ml of 10-fold dilutions of faecal samples or swab specimens were cultured on sheep blood agar (Oxoid Ltd, Basingstoke, UK) Mannitol salt agar (Oxoid) and MacConkey 1 agar (Oxoid) for 2 days at 37°C, Sabouraud dextrose agar (BBL, Becton-Dickinson and Co., Cockeysville, MD, USA) for 7 days at 37°C and Sabouraud dextrose agar (Becton-Dickinson) for 7 days at 30°C. For quantitative anaerobic culture 10-fold dilutions of faecal and throat-swab specimens were cultured on 5% horse blood agar (Oxoid) supplemented with haemin/menadione for 7 days at 37°C under anaerobic conditions. The identification of microorganisms at the species level was performed by standard microbiological techniques. For the determination of anaerobic bacteria, subcultures were made (50-100 colonies) for aerobic and anaerobic incubation and gram staining was performed. The results for *Staphylococcus aureus*, aerobic Gram-negative bacilli, yeasts, anaerobic Gram-negative and Gram-positive bacilli were reported as the number of colony-forming units per gram faeces (CFU/g) or per millilitre for throat-swab specimens (CFU/ml). For throat swabs, the number of VG streptococci was determined (CFU/ml). The antimicrobial activity of levofloxacin on *S. aureus*, VG streptococci and Gram-negative bacilli was tested by the determination of the minimal inhibitory concentration (MIC) by E-test (AB Biodisk, Solna, Sweden). Breakpoints for levofloxacin were defined according to NCCLS standards, as susceptible (MIC $\leq$2 mg/ml), intermediate resistant (MIC = 4 mg/l) and resistant (MIC $\geq$8.0 mg/l).

Pharmacokinetic methods

The assessment of pharmacokinetic parameters of levofloxacin was performed in 10 subjects (50%), being the first cohort of 10 patients enrolled in the study. The pharmacokinetic study consisted of two phases. During phase 1, samples (7 ml) of venous blood were collected from an indwelling central venous catheter, immediately prior to the administration of the first dose of levofloxacin, and at 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 12.0, 18.0 and 24.0 h after administration. Phase 2 was initiated as soon as the patient was profoundly granulocytopenic (ANC $<0.5 \times 10^9$/l). Venous samples were drawn, immediately prior to dosing and at 1.0, 4.0, 8.0, 18.0 and 24.0 h after dosing. All samples were collected in standard tubes and centrifuged after clotting. The serum was separated and stored at -20°C until analysis. To investigate whether

levofloxacin undergoes in vivo enantioconversion from S-(-)-ofloxacin (levofloxacin) to R-(+)-ofloxacin, urine samples were collected from each patient within 10 h after the administration of the first oral dose of levofloxacin. Urine samples were stored at -20°C.

Venous blood samples were assayed by reversed phase high-pressure liquid chromatography with diode array detection (Gynkotek, Germering, Germany). Separations were carried out on a 3.9 × 150 mm^2 Symmetry C_{18} column (Waters, Milford, MA, USA), using ciprofloxacin as an internal standard. The mobile phase was 25 mM phosphate buffer (pH 3.0): acetonitrile (85:15). Chromatography was performed at ambient temperature and a flow rate of 1.0 ml/min. Detection wavelength was at 290 nm. The analysis of the urine samples was carried out on a Beckman P/ACE 5500 capillary electrophoresis system (Beckman Instruments, Fullerton, CA, USA) equipped with a diode array detector, using 0.35 mM sulphated *β*-cyclodextrin as a chiral selector. Detection wavelength was at 291 nm.

From the data obtained, the apparent total body clearance (CL/*F*), apparent volume of distribution at steady state (V_{ss}/F), maximum concentration in the serum (C_{max}), time to maximum concentration in the serum (T_{max}) and area under the curve for the 24-h dosing interval (AUC_{0-24}) of levofloxacin were determined. Patients were tested for differences between phase 1 and 2 pharmacokinetics, with each patient being its own control. Pharmacokinetic calculations were performed with the MW/Pharm software (MediWare, Groningen, The Netherlands), which is capable of curve fitting and simulations according to a one-, two- or three-compartment model, and provides compartmental and noncompartmental pharmacokinetic parameters. An extravascular, two-compartment model with lag time was found to best describe the data.

Statistical analysis

Changes in the number of CFUs of anaerobes in faecal samples and swab specimens over time were analysed by regression analysis. The regression coefficient (*B*) and its 95% confidence interval (CI) were calculated. Subsequent analysis of variance was performed to assess goodness-of-fit of the line. A paired Student's *t*-test was used for the statistical analysis of differences in mean pharmacokinetic parameters between phases 1 and 2. All tests were two-sided and a *P*-value less than 0.05 was considered statistically significant.

Results

Patient characteristics

In all, 22 patients were enrolled in the study, two of which were excluded from analysis. One patient was not evaluable because he erroneously received concomitant antibacterial prophylaxis with trimethoprim-sulphamethoxazole, the other patient died a week after entry from cerebral haemorrhage due to thrombocytopenia. Characteristics of the remaining 20 evaluable patients are given in Table 1. All patients tolerated the study drug well, no adverse effects occurred, which could be attributed to levofloxacin. The mean number of levofloxacin doses received per patient was 22, no missed doses were reported.

Table 1. Patient characteristics.

Variable	Number of patients (%) unless otherwise specified
General characteristics	
Number of patients	20
Male	13 (65)
Female	7 (35)
Age (years, mean ± SD)	52.1 ± 15.5
Hospital stay (days, mean ± SD)	25.7 ± 5.3
Disease	
AML	7 (35)
ALL	2 (10)
Myelodysplastic syndrome	1 (5)
Lymphoma	4 (20)
Multiple myeloma	6 (30)
Treatment	
Stem cell transplant	11 (55)
Ara-C containing regimen	7 (35)
Duration of neutropenia[a]	
ANC <0.1 (days, mean ± SD)[b]	19.9 ± 5.6
ANC <0.5 (days, mean ± SD)	21.6 ± 5.8
Study drug	
Administrations per patient (mean ± SD)	22.0 ± 5.9
Administrations per patient (range)	13 - 36

[a] Duration of neutropenia is calculated from the start of chemotherapy.

[b] ANC = absolute neutrophil count (x 10^9/l)

Microbiological results

During the course of the study, a total of 137 throat swabs (mean 6.9/patient) and 115 faecal samples (mean 5.8/patient) were collected. In all, 17 anal swabs had to be taken because the patient was not able to produce a stool specimen. Culture data of the throat-swab specimens are summarized in Table 2. VG streptococci persisted from the start of chemotherapy throughout the duration of the neutropenic episode. At day 0-1, VG streptococci were highly susceptible to levofloxacin in 19 patients (MIC ≤1mg/l), in one patient an intermediate-susceptible strain was found (day 2, MIC =4 mg/l). During the prophylactic administration of levofloxacin in four patients, VG streptococci were isolated with MIC values increasing in one step up to ≥32 mg/l, this occurred, respectively, after 11, 13, 14 and 21 days of treatment. These patients received additional prophylaxis with oral penicillin, 250 mg four times daily.
MIC values of VG streptococci that remained susceptible to levofloxacin (MIC ≤2 mg/l) were analysed separately, leaving resistant strains out of the calculations. Even in these susceptible strains there was a gradual but significant increase of MIC values during the study period (regression coefficient B =0.03, CI_{95} 0.01-0.04, P =0.03).

Table 2. Culture data of throat-swab specimens.

	Days on study				
	0-1	2-7	8-14	15-21	>22
Number of subjects	20	20	20	17	6
Viridans group streptococci (n, %)	19 (95)	16 (80)	15 (75)	9 (53)	2 (33)
MIC (median, mg/l)	0.75	0.50	0.75	2.0	0.5
MIC (range, mg/l)	0.5 - 1.0	0.38 - 4.0	0.38 - ≥32	0.125 - ≥32	0.125 - 2.0
MIC ≥8 mg/l (n)	0	0	3	3	0
β-haemolytic streptococci (n, %)	0	1 (5)	0	0	0
MIC (mg/l)		0.38			
Staphylococcus aureus (n, %)	1 (5)	0	0	0	0
MIC (mg/l)	0.064				
Gram-negative microorganisms	0	0	0	0	0
Candida spp (n, %)	2 (10)	2 (10)	2 (10)	2 (12)	0

MIC = 4 mg/l indicates intermediate susceptibility, MIC ≥8 mg/l indicates resistance to levofloxacin.

Culture data of faecal samples are given in Table 3. On admission 18/20 (90%) of patients were colonized with *Enterobacteriaceae* or other Gram-negative microorganisms (CFU $>10^3$/g faeces), in two patients *S. aureus* was identified (CFU $>10^3$/g faeces). Successful

eradication of these microorganisms was achieved within 5 days of levofloxacin administration. Two patients were colonized with a levofloxacin resistant *Escherichia coli* on admission (MIC ≥32 and 12 mg/l, respectively). In a third patient, a levofloxacin-resistant *Escherichia coli* (MIC ≥32 mg/l) was isolated from a stool specimen during the second week of his admission (day 13). In addition to levofloxacin, these three patients received oral colistin, 300 mg three times daily, which provided adequate eradication of levofloxacin-resistant *E. coli*. In one patient an *Acinetobacter lwoffii* was isolated on day 6 and in another patient a *Sphingomonas paucimobilis* was found on day 12. Both strains were considered transient flora because they proved to be susceptible to levofloxacin and were isolated only once.

Table 3. Culture data of faecal samples and anal-swab specimens.

	Days on study				
	0-1	2-7	8-14	15-21	>22
Number of subjects	20	20	20	17	6
Enterobacteriaceae					
Escherichia coli (n, %)	15 (75)	5 (25)	1 (6)	0	0
MIC (median, mg/l)	0.032	0.23	≥32		
MIC (range, mg/l)	0.008 - ≥32	0.016 - ≥32			
MIC >8 mg/l (n)	2	2	1[a]	0	
Klebsiella pneumoniae (n, %)	2 (10)	0	0	0	0
MIC (mean, mg/l)	0.262				
Enterobacter cloacae (n, %)	1 (5)	0	0	0	0
MIC (mg/l)	0.04				
Proteus penneri (n, %)	1 (5)	0	0	0	0
MIC (mg/l)	0.032				
Hafnia alvei (n, %)	1 (5)	0	0	0	0
MIC (mg/l)	0.008				
S. aureus (n, %)	2 (10)	0	0	0	0
MIC (mean, mg/l)	0.110				
Sphingomonas paucimobilis	0	0	1 (5)	0	0
MIC (mg/l)			2.0		
Acinetobacter lwoffii	0	1 (5)	0	0	0
MIC (mg/l)		0.25			
Non-ferment. Gram neg. rod (n, %)	1 (5)	0	0	0	0
MIC (mg/l)	0.25				
Candida spp (n, %)	5 (25)	6 (30)	11 (55)	6 (35)	2 (33)

MIC ≥8 mg/l indicates resistance to levofloxacin.

[a] patient colonized with a resistant E-coli (MIC ≥32 mg/l) on day 13.

Quantitative anaerobic cultures of throat-swab specimens obtained at baseline showed a mean number of 6.2×10^6 CFU/ml (range 3.0×10^6-7.4×10^6 CFU/ml). A predominance of anaerobic Gram-positive bacilli was found in throat samples, subdivided into cocci (21%) and rods (49%). In faecal samples obtained at baseline, a mean number of anaerobic bacilli of 1.6×10^9 CFU/g (range 1.4×10^5-1.3×10^{10} CFU/g) was found, which were mainly Gram-negative rods (90%). No significant changes of colonization rates of anaerobes over time were observed, as expressed by the total number of CFU/g faeces (B=0.02, CI_{95} -0.01-0.05, P=0.1) or per ml throat-swab specimen (B=-0.01, CI_{95} -0.04-0.10, P=0.3). Moreover, there were no significant shifts from predominance of Gram-positive to Gram-negative anaerobic microflora or vice versa, during levofloxacin prophylaxis.

Fever, defined as a sustained axillary temperature of >38.5°C, occurred in eight (40%) patients. Probable causes of fever were a clinically documented infection of the lung (n=2), an infection of the skin with *Absidia* spp (n=1) and fever of unknown origin (n=1). Four patients developed blood-stream infections with Gram-positive bacteria, including coagulase-negative staphylococcus (n=1), *Enterococcus faecalis* (n=1), *S. oralis* (n=1) and *E. faecium* + coagulase-negative staphylococcus (n=1). All isolated microorganisms displayed high level resistance to levofloxacin (MIC ≥32). Patients with fever received imipenem-cilastatin as initial empirical antibiotic treatment. In two patients vancomycin was added, because of imipenem-resistant pathogens (*E. faecium,* coagulase-negative staphylococcus). In another two patients imipenem-cilastatin was switched to vancomycin in combination with aztreonam, because of an allergic skin reaction possibly due to imipenem. Amphotericin B was given to two patients, one with a proven infection with *Absidia* spp and another because of persisting fever after 4 × 24 h of treatment with imipenem. All patients with fever recovered without significant sequelae. There was no mortality attributable to infectious complications.

Pharmacokinetic data

Urine samples were collected in 10 patients. No in vivo enantioconversion from S-(-)-ofloxacin (levofloxacin) to R-(+)-ofloxacin was observed. For this reason, it was not necessary to use an enantio-selective separation method for the analysis of the blood samples.

A total of 170 venous blood samples were drawn in 10 patients. In all, 10 samples per patient were collected during the 24 h period following first dose administration (phase 1), seven samples per patient were obtained immediately after neutropenia had been established (phase 2). Phase 2 sampling was initiated at a mean of 13.9±3.8 days from first dose administration.

At that time all patients were neutropenic (ANC <0.5 × 10^9/l), 8/10 (80%) of patients had a total leucocyte count of ≤0.1 × 10^9/l. The mean concentrations found in the serum following the first dose of 500 mg levofloxacin (phase 1) and at neutropenia (phase 2) are shown in Figure 1. The derived mean ± SD pharmacokinetic parameters are listed in Table 4.There was no statistically significant difference between the mean area under the concentration-time curve at first dose (AUC_{0-24}) and during neutropenia (AUC_{0-24} multiple doses at steady state) (P >0.05). Also no statistically significant differences were found in mean values of the maximum concentration of levofloxacin in the serum (C_{max}), the time to maximum concentration in the serum (T_{max}), the apparent volume of distribution at steady state (V_{ss}/F) and the apparent total body clearance (CL/F) (P >0.05). These data indicated that the systemic availability of levofloxacin and its serum concentration-time profile were equivalent for administration at day 1 and during neutropenia.

Table 4. Pharmacokinetic parameters of levofloxacin in the first cohort of 10 study patients, at baseline and during neutropenia (neutrophil count <0.5 x 10^9/l).

	C_{max} (µg/ml)	T_{max} (h)	AUC_{0-24} (h·µg/ml)	V_{ss}/F (Liters)	V_{ss}/F (Liters/kg)	CL/F (ml/min)	CL/F (ml/min/kg)
First dose (mean ± SD)	6.74 ± 1.76	1.18 ± 0.49	66.1 ± 19.9	126 ± 36	1.65 ± 0.36	157 ± 54	2.04 ± 0.51
Neutropenia (mean ± SD)	7.02 ± 1.96	1.36 ± 0.54	58.2 ± 23.6	129 ± 48	1.73 ± 0.81	158 ± 60	2.08 ± 0.80
Mean difference	-0.28	-0.18	7.94	-3	-0.08	-1	-0.04
95% CI of the mean difference	-2.03-1.46	-0.54-0.18	-0.23-16.11	-43,0-37,0	-0.70-0.54	-22.0-20,0	-0.34-0.26
P (2-tailed)	0.72	0.28	0.06	0.87	0.78	0.93	0.78

C_{max} maximum concentration in serum, T_{max}, time to maximum concentration in serum, AUC_{0-24} area under the curve for the 24-h dosing interval, V_{ss}/F apparent volume of distribution at steady state, CL/F apparent total body clearance.

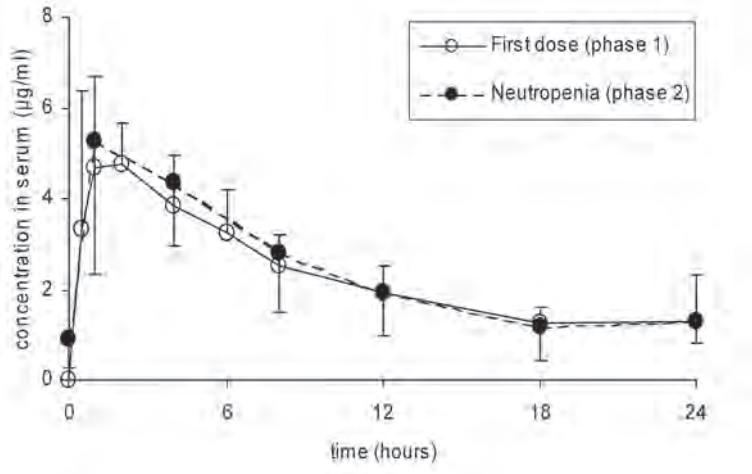

Figure 1. Profiles of mean levofloxacin concentrations (error bars represent ± SD) in serum of 10 patients, following the first oral administration of 500 mg levofloxacin and after multiple dosing during neutropenia (neutrophil count <0.5 x 10^9/l).

Discussion

Levofloxacin is a new fluoroquinolone, with increased activity against Gram-positive bacteria, yet it retains good activity against *Enterobacteriaceae* and other Gram-negative microorganisms. The present study was conducted to resolve two major issues with regard to the use of levofloxacin as antibacterial prophylaxis in neutropenic subjects. First, to assess the effect of multiple dose administration of levofloxacin on gastrointestinal microflora and second, to investigate its pharmacokinetic profile during neutropenia.

As expected, levofloxacin provided excellent eradication of *Enterobacteriaceae* within 5 days from the start of antibacterial prophylaxis. In line with the preclinical data, the median MIC values of levofloxacin for *E. coli*, *Klebsiella* spp and *Enterobacter* spp were very low, except for two patients who were already colonized with a levofloxacin-resistant *E. coli* on admission (MIC ≥32 and 12 mg/l, respectively). In addition, one patient appeared to be colonized with a resistant *E. coli* during the second week of his admission. Resistance of *E. coli* to levofloxacin is uncommon. However, the systematic use of ciprofloxacin prophylaxis in neutropenic subjects has been clearly associated with the emergence of quinolone-resistant *E. coli*.[17-20] Our observation indicates that levofloxacin may be expected to induce resistance in Gram-negative microorganisms to a comparable extent as ciprofloxacin. We used additional prophylaxis with oral colistin in these patients. This resulted in the eradication of

the levofloxacin-resistant strains. In a recent publication, the addition of colistin to ciprofloxacin appeared to reduce the emergence of quinolone-resistant Gram-negative bacteria and to prevent Gram-negative sepsis in neutropenic patients.[18] In our patients no systemic infections with Gram-negative bacteria occurred.

Colonization with VG streptococci was reduced by the prophylactic administration of levofloxacin. Of concern was the increase of MIC values for streptococci over time. In four patients VG streptococci with MICs of ≥32 mg/l were isolated during the course of antibacterial prophylaxis. In addition, even for VG streptococci that remained susceptible to levofloxacin, a significant gradual increase of MIC values was found during the study period. In vitro studies have demonstrated high susceptibility of VG streptococci to levofloxacin, with MIC values ranging from 0.25 to 1 mg/l.[21,22] Our data indicate that resistance of VG streptococci may develop during long-term administration of levofloxacin. This observation is in line with recently published clinical data.[23] In a series of 37 neutropenic patients receiving levofloxacin prophylaxis, bacteraemia with VG streptococci occurred in six (16%) patients, three of whom developed septic shock. All isolates displayed diminished susceptibility to levofloxacin and cross-resistance to other quinolones. The authors conclude that the prophylactic administration of levofloxacin may result in the selection of quinolone-resistant VG streptococci.

Four patients in our study developed blood-stream infections with Gram-positive bacteria, including coagulase-negative staphylococci, *E. faecalis*, *S. oralis* and *E. faecium*. Preclinical data show that most strains of enterococci and coagulase-negative staphylococci are susceptible to levofloxacin, although MIC values are higher than for VG streptococci. MIC values are reported to range from 0.13 to 4 mg/l for coagulase-negative staphylococci and from 0.25 to ≥32 mg/l for enterococci.[21,22] In a study on haemato-oncology patients in Switzerland, 65% of strains of coagulase-negative staphylococci were susceptible to levofloxacin and even 100% of strains of enterococci.[24] Despite these compelling data, our findings indicate that during levofloxacin prophylaxis breakthrough infections with coagulase-negative staphylococci or enterococci may occur.

Levofloxacin did not affect the anaerobic component of the gut microflora. The number of anaerobic microorganisms, expressed as the number of CFU/g faeces or per ml throat-swab specimen, did not change significantly during the course of the prophylactic regimen. This may be of crucial importance in view of preservation of colonization resistance, which is based on the concept that selective suppression of the aerobic Gram-negative bacteria in the

digestive tract, without the impairment of the anaerobic flora, prevents the adherence, proliferation and invasion of potentially pathogenic bacterial and fungal species.[25]

Our study is the first to report pharmacokinetic data of levofloxacin in neutropenic patients. The estimated mean values for C_{max}, T_{max}, $AUC_{(0-24)}$ and CL/F in patients with haematological malignancies were well comparable with those determined in healthy subjects.[13,26] In addition, we showed that individual pharmacokinetic parameters calculated after the first oral administration of levofloxacin were not statistically different from those calculated at neutropenia. This is important because significant changes in the pharmacokinetic profile may occur in patients receiving intensive chemotherapy. Mucosal damage, for example, may either decrease or increase the rate of antibiotic absorption, shifts and decreases in the serum albumin may significantly affect the volume of distribution of the drug. It has been shown previously that the absorption of oral ciprofloxacin and to a lesser extent of oral ofloxacin are reduced following cytotoxic chemotherapy.[15,16] Pharmacokinetics of levofloxacin appear to be unaffected by the administration of chemotherapy or neutropenia, indicating that on these occasions there is no need for dose adjustment.

In conclusion, levofloxacin, administered orally as antibacterial prophylaxis to patients receiving intensive chemotherapy for haematological malignancies, provides adequate eradication of Gram-negative microorganisms and *S. aureus* and preserves the anaerobic component of the bowel flora. Moreover, the pharmacokinetic properties of levofloxacin are not altered due to chemotherapy or neutropenia. Levofloxacin is easily administered once daily and well tolerated. Of concern, however, is the increasing resistance of VG streptococci during prolonged administration of levofloxacin and a diminished susceptibility of coagulase-negative staphylococci and enterococci. These findings may hamper its use as a single agent for prophylaxis in neutropenic subjects. This pilot study, however, with a limited number of subjects does not allow for definite conclusions on that issue. Hence, the question remains open as to how levofloxacin prophylaxis compares with standard prophylactic regimens as for tolerability, efficacy, induction of resistance and the number of break-through infections. To address these questions, we are currently investigating levofloxacin vs ciprofloxacin plus oral penicillin in a randomized clinical trial.

Acknowledgements

This study was supported in part by a grant from Aventis Pharma BV, Hoevelaken, The Netherlands.

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Chapter 4

Levofloxacin versus ciprofloxacin plus phenethicillin for the prevention of bacterial infections in patients with haematological malignancies.

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Clinical Microbiology and Infection 2007;13:497-503.

Abstract

An open-label randomized clinical trial was designed to compare the efficacy and tolerance of levofloxacin and ciprofloxacin plus phenethicillin for the prevention of bacterial infections in patients with high-risk neutropenia, and to monitor the emergence of antimicrobial resistance. Adult patients ($n = 242$) scheduled to receive intensive treatment for haematological malignancies were assigned randomly to receive oral prophylaxis with either levofloxacin 500 mg once-daily ($n = 122$), or ciprofloxacin 500 mg twice-daily plus phenethicillin 250 mg four-times-daily ($n = 120$). The primary endpoint was failure of prophylaxis, defined as the first occurrence of either the need to change the prophylactic regimen or the initiation of intravenous broad-spectrum antibiotics. This endpoint was observed in 89 (73.0%) of 122 levofloxacin recipients and in 85 (70.8%) of 120 ciprofloxacin plus phenethicillin recipients (RR 1.03, CI_{95} 0.88-1.21, $P = 0.71$). No differences were noted between the two groups with respect to secondary outcome measures, including time to endpoint, occurrence of fever, type and number of microbiologically documented infections, and administration of intravenous antibiotics. A questionnaire revealed that levofloxacin was tolerated significantly better than ciprofloxacin plus phenethicillin. Surveillance cultures indicated the emergence of viridans group (VG) streptococci resistant to levofloxacin in 17 (14%) of 122 levofloxacin recipients; in these cases, the prophylactic regimen was adjusted. No bacteraemia with VG streptococci occurred. It was concluded that levofloxacin and ciprofloxacin plus phenethicillin are equally effective in the prevention of bacterial infections in neutropenic patients, but that levofloxacin is tolerated better. Emergence of levofloxacin-resistant VG streptococci is of concern, but appears to be a manageable problem.

Introduction

Quinolones have been used extensively in the past two decades for the prevention of bacterial infections during neutropenia in cancer patients. It is evident that this approach reduces the incidence of Gram-negative bacteraemia significantly.[1-3] Unfortunately, this is counterbalanced by an increased incidence of infections with Gram-positive bacteria. In particular, viridans group (VG) streptococci and coagulase-negative staphylococci have emerged as a frequent cause of morbidity and mortality.[4,5] To overcome this problem, quinolone prophylaxis has been combined with other antimicrobial agents, e.g., penicillin, macrolides and vancomycin, that are active against Gram-positive cocci.[6-9]

An alternative approach could involve the use of new-generation quinolones, which are more potent against Gram-positive pathogens. Levofloxacin, as a representative of this group, has been reported to reduce the incidence of fever and other infection-related outcomes in neutropenic cancer patients, compared with a placebo,[2,10,11] but important issues remain to be addressed. First, no data are available from controlled clinical trials that allow a direct comparison between the use of levofloxacin prophylaxis and the use of ciprofloxacin plus an antibiotic with anti-Gram-positive bacteria activity. Second, there have been some alarming reports concerning the emergence of levofloxacin-resistant Gram-positive microorganisms, in particular VG streptococci.[12,13]

During the past few years, patients admitted to the haematology department of the VU University Medical Center, Amsterdam, The Netherlands have received ciprofloxacin plus phenethicillin for the prevention of bacterial infections during neutropenia as the standard of care. When levofloxacin became available in The Netherlands, the randomized clinical trial described in this study was conducted to compare levofloxacin with ciprofloxacin plus phenethicillin with respect to their efficacy as antibacterial prophylaxis for neutropenic patients. In addition, the trial was designed to investigate the tolerance of these compounds and to closely monitor emerging antimicrobial resistance.

Patients and methods

Patients

Consecutive adult patients with a haematological malignancy who were hospitalized at the haematology department of the VU University Medical Center for high-dose combination

chemotherapy, with or without autologous or allogeneic haematopoietic stem-cell transplantation, were eligible for this study. An anticipated granulocytopenic period (granulocytes $<0.5 \times 10^9$/L) of ≥10 days was required. Patients were enrolled only once. Exclusion criteria were active infection or antibacterial therapy at entry, a history of hypersensitivity to fluoroquinolones, a creatinine clearance of <15 mL/min, or elevation of transaminases to greater than three-fold the normal upper limit. The protocol was approved by the institutional scientific and ethical committees, and all participants provided written informed consent.

Randomization and prophylactic regimen

The study was a prospective, single-centre, open-label, randomized clinical trial. Patients were assigned randomly by consecutively drawn, sealed envelopes to receive either levofloxacin 500-mg tablets once-daily or ciprofloxacin 500-mg tablets twice-daily, plus, from day 7 after the start of chemotherapy, phenethicillin 250-mg tablets four-times-daily. Prophylaxis was begun on the first day of chemotherapy and was continued until recovery to a granulocyte count of $>0.5 \times 10^9$/L. Phenethicillin was initiated on day 7 because, from that time on, oropharyngeal mucositis was to be expected, and this has been identified as an independent risk-factor for infections with streptococci.[4] Compliance was monitored by counting tablets. In addition to the study medication, all patients received fluconazole 50 mg once-daily and 2 mg nasal amphotericin B spray three-times-daily. A central venous catheter was inserted before the start of chemotherapy.

Clinical and microbiological evaluation of subjects

Randomized patients were examined daily for clinical signs of infection. Surveillance cultures for identification of colonizing bacteria and yeasts were taken from throat and anus before the first dose of the study drugs and once-weekly thereafter. If appropriate, the prophylactic regimen was adjusted according to the resistance patterns of the microorganisms identified.

If the axillary temperature increased to >38.5°C, or if other signs or symptoms of an infection occurred without fever, clinical evaluation was performed according to a local protocol, including a complete physical examination, a chest X-ray, and appropriate samples for microbiological cultures. At least two separate blood samples were obtained for culture, from both the central venous catheter and from a peripheral vein. Subsequently, empirical antibiotic therapy was initiated, consisting of intravenous imipenem-cilastatin 500 mg four-times-daily. If the fever did not resolve in ≤96 h, patients received antifungal therapy. In case of initiation

of broad-spectrum antibacterial therapy, levofloxacin or ciprofloxacin were continued, but phenethicillin was discontinued.

Pathogenic microorganisms, isolated either from surveillance cultures or from cultures obtained from patients with presumed infection, were identified to the species level by standard microbiological techniques. MICs of levofloxacin and ciprofloxacin for staphylococci, streptococci and Gram-negative bacilli were determined by E-tests (AB Biodisk, Solna, Sweden). Susceptibility of streptococci to phenethicillin was determined by disk-diffusion tests and was reported as susceptible, intermediately-resistant or resistant. Breakpoints were defined according to CLSI standards.

Tolerance of the study medication and toxicity

Patients were asked to complete a questionnaire, which recorded a 'tolerance score' for the study medication on a daily basis. Tolerance of the study drug was classified as 'not able to take the study drug', 'difficult intake', 'minor problems on intake', or 'intake without any problem'. Any adverse event that was possibly or probably related to the study medication was recorded. Routine clinical chemistry tests were performed weekly, and any deterioration in liver enzymes, bilirubin or kidney function was recorded. All adverse events were classified using the Common Terminology Criteria for Adverse Events v.3.0 (CTCAE; National Cancer Institute, Bethesda, MD, USA). Following an adverse event, study medication was either continued or discontinued, according to the judgement of the responsible physician.

Outcome

The primary outcome measure of the study was success or failure of the prophylactic regimen. Failure of prophylaxis was a composite endpoint, defined as the need to change the prophylactic regimen for any reason, or the initiation of broad-spectrum antibacterial therapy, whichever event occurred first. The primary endpoint was chosen to reflect the effects of the prophylactic regimens on the most relevant clinical events. Furthermore, the study was designed to include patient follow-up beyond this first event, and data were analysed on an intention-to-treat basis. Secondary outcome measures were the time to primary endpoint, the occurrence of fever, the type and number of documented infections, the use of antimicrobial agents, and the tolerance of the study drug. Moreover, the study design provided close monitoring of the acquisition of antimicrobial resistance by the pathogens isolated.

Statistical evaluation

It was estimated from previous studies in the same ward that *c.* 30% of patients survive the neutropenic episode without requiring change of prophylaxis or initiation of broad-spectrum antibiotics. Thus, according to the definitions used in the present study, 70% of patients were expected to experience failure of prophylaxis. Sample size was calculated to detect a 25% reduction (from 70% to 52%) in failure of prophylaxis. To detect such a difference with a significance level (α) of 0.05 (two-tailed) and a statistical power of 80%, 120 patients per arm were required. Differences between groups in categorical variables were analysed with the chi-square test. In case of variables with an ordering or grading scale, the chi-square test for trend was used. The relative differences between the groups were also expressed as relative risks (RRs) with 95% confidence intervals (CI_{95}). The Mann-Whitney non-parametric *U*-test was used for comparison of means. Differences in survival without failure of prophylaxis were assessed by the log-rank test, and Kaplan-Meier curves were plotted for each study group.

Results

Between January 2002 and July 2005, 245 patients were enrolled in the study. Three patients were excluded from analysis. One patient withdrew informed consent, another patient was erroneously enrolled twice, and one patient died on the day of randomization because of disease progression. Of the 242 evaluable patients, 122 were assigned to receive levofloxacin and 120 to receive ciprofloxacin and phenethicillin. Basic patient characteristics of the two treatment groups are listed in Table 1. No significant differences were found in gender, age, type and remission status of the haematological disease and treatment variables, including stem-cell transplantation procedures. However, the neutropenic episode was significantly longer in the ciprofloxacin-phenethicillin group (mean difference to a granulocyte count of $>0.5 \times 10^9$/L = 1.4 days, $P = 0.044$; mean difference to granulocyte count $>0.1 \times 10^9$/L = 1.6 days, $P = 0.017$). Patients receiving levofloxacin remained in hospital for a mean of 25.5 days, compared with 28.1 days for patients receiving ciprofloxacin-phenethicillin, but this was not statistically significant (mean difference 2.6 days, $P = 0.13$).

Table 1. Patient characteristics and clinical course variables.

	Levofloxacin		**Ciprofloxacin**	
	n	%	*n*	%
Total patients	122	50.4	120	49.6
Age, years				
Median (range)	55 (18-71)		54 (19-71)	
Gender				
Male	76	62.3	79	65.8
Female	46	37.7	41	34.2
Diagnosis				
ALL	10	8.2	10	8.3
AML	19	15.6	28	23.3
Multiple Myeloma	46	37.7	39	32.5
Lymphoma	35	28.7	34	28.4
Myelodysplasia	4	3.3	3	2.5
Other	8	6.5	6	5.0
Disease status				
Newly diagnosed	81	66.4	79	65.8
Recurrent	41	33.6	41	34.2
Remission status				
Active disease	21	17.2	27	22.5
Partial remission	70	57.4	59	49.2
Complete remission	31	25.4	34	28.3
Treatment variables				
First course or induction	15	12.3	16	13.3
Second course or consolidation	21	17.2	26	21.7
Stem-cell transplantation	86	70.5	78	65.0
autologous stem-cells	74	86.0	65	83.3
allogeneic stem cells	12	14.0	13	16.7
Laminar airflow room	13	10.7	16	13.3
Haematopoietic growth factor (G-CSF)	10	8.2	8	6.7
Duration of neutropenia (ANC < 0.5 x 10^9/L), days				
Mean[a]	21.8		23.2	
Median (range)	20.5 (15-46)		22.0 (12-55)	
Duration of severe neutropenia (ANC < 0.1 x 10^9/L), days				
Mean[b]	19.9		21.5	
Median (range)	18.0 (13-46)		20.0 (11-53)	
Hospital stay, days				
Mean	25.5		28.1	
Median (range)	23.0 (16-58)		25.0 (14-155)	

All differences not significant (NS), except a $P = 0.044$, b $P = 0.017$.

Failure of prophylaxis and febrile episodes

The primary endpoint 'failure of prophylaxis' was observed in 89 (73.0%) of 122 patients receiving levofloxacin, compared with 85 (70.8%) of 120 patients receiving ciprofloxacin-phenethicillin (RR 1.03, CI_{95} 0.88-1.21, P = 0.71) (Table 2). The distribution of events responsible for failure of prophylaxis; change of the prophylactic regimen and initiation of intravenous antibiotics, were similar in the two groups. The time to failure of prophylaxis (Table 2), and Kaplan-Meier estimates of the proportion of patients surviving without failure of prophylaxis (Figure 1), showed no clear advantage for either prophylactic regimen.

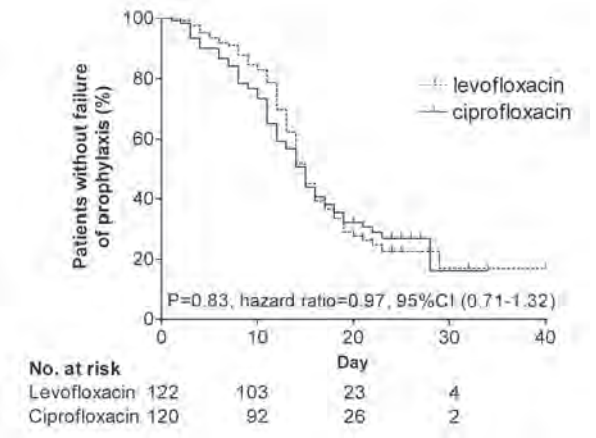

Figure 1. Kaplan-Meier estimates of survival without failure of prophylaxis. Differences between the two treatment groups were analyzed by the log-rank test and by computing the hazard ratio with its 95% confidence interval.

In the intention-to-treat analysis, 28 (23.0%) of 122 patients in the levofloxacin group needed adjustment of the prophylactic regimen during the study period, compared with 39 (32.5%) of 120 patients in the ciprofloxacin-phenethicillin group (RR 0.71, CI_{95} 0.47-1.07, P = 0.07). The main reason for change of prophylaxis in the levofloxacin group was decreased susceptibility or resistance of bacterial isolates, as indicated by surveillance culture data. Intolerance of the study medication was the most common reason for change of the prophylactic regimen in patients receiving ciprofloxacin-phenethicillin. This event occurred significantly less frequently in levofloxacin recipients (RR 0.11, CI_{95} 0.03-0.46, P = 0.0002).
The occurrence and duration of fever, and the number of patients receiving broad-spectrum intravenous antibiotics, were similar in both groups. Furthermore, there were no significant differences in either type or number of antibacterial and antifungal antibiotics administered.

Table 2. Administration of study drug and outcome

	Levofloxacin		**Ciprofloxacin**		Relative risk	
	n	%	n	%	(95%CI)	*P*
Total patients	n = 122		n = 120			
Primary endpoint	89	73.0	85	70.8	1.03 (0.88-1.21)	0.71
Change of prophylaxis (as first event)	27	22.2	33	27.5	0.80 (0.52-1.25)	0.33
Initiation i.v. antibiotics (as first event)	62	50.8	52	43.3	1.17 (0.90-1.54)	0.24
No endpoint (censored)	33	27.0	35	29.2	0.93 (0.62-1.39)	0.71
Time to endpoint, days						
Mean ± SD	15.2 ± 6.2		14.5 ± 6.8			
Median (range)	15.0 (2-40)		14.5 (1-34)			
Fever	68	55.7	64	53.3	1.05 (0.83-1.32)	0.71
Duration of fever, days						
Mean ± SD	5.9 ± 4.9		6.1 ± 4.3			0.85
Median (range)	5.0 (2-36)		5.0 (1-19)			
Change of prophylaxis (any event)	28	23.0	39	32.5	0.71 (0.47-1.07)	0.07
Allergic reaction	3	2.5	9	7.5	0.33 (0.09-1.18)	0.07
Surveillance culture data	22	18.0	12	10.0	1.80 (0.94-3.48)	0.07
Intolerance of study drugs	2	1.6	18	15.0	0.11 (0.03-0.46)	0.0002
Elevated liver enzymes	1	0.8	0	0.0		0.32
Initiation i.v. antibiotics (any event)	73	59.8	69	57.5	1.04 (0.84-1.29)	0.71
Temperature rise > 38.5 °C	66	54.1	60	50.0	1.08 (0.85-1.38)	0.52
Suspected infection, without fever	7	5.7	9	7.5	0.77 (0.29-1.99)	0.58
Days with intravenous antibiotics, days						
Mean ± SD	9.2 ± 7.1		10.9 ± 7.4			
Median (range)	7.0 (2-38)		8.0 (2-35)			
Intravenous antibiotics						
Imipenem-cilastatin	71	58.2	65	54.2	1.07 (0.86-1.34)	0.53
Vancomycin	27	22.1	22	18.3	1.21 (0.73-2.00)	0.46
Ceftazidim	7	5.7	7	5.8	0.98 (0.36-2.72)	0.97
Other antibacterial	5	4.1	7	5.8	0.70 (0.23-2.15)	0.53
Itraconazole	17	13.9	23	19.2	0.73 (0.41-0.15)	0.27
Other antifungal	7	5.7	6	2.5	1.15 (0.40-3.32)	0.80
Adverse events						
skin rash	16	13.1	15	12.5	1.05 (0.54-2.03)	0.89
elevated liver enzymes	4	3.3	3	2.5	1.31 (0.30-5.74)	0.72
Mortality	2	1,6	4	3,3	0.50 (0.09-2.68)	0.41
Death due to infection	1	0,8	3	2,5	0.33 (0.03-3.15)	0.31
Death from non-infectious causes	1	0,8	1	0,8	0.98 (0.06-15.6)	0.99

The number of days (mean ± SD) for which patients received intravenous antibiotics was 9.2 ± 7.1 in the levofloxacin group compared with 10.9 ± 7.4 in the ciprofloxacin-phenethicillin group. The difference of 1.7 days in favour of the levofloxacin group almost reached statistical significance ($P = 0.051$).

Infections

The number of microbiologically documented infections was similar in both groups, with 21 (17.2%) of such infections observed in levofloxacin recipients and 22 (18.3%) in ciprofloxacin-phenethicillin recipients (RR 0.94, CI_{95} 0.55-1.62, $P = 0.82$) (Table 3). Most patients with a microbiologically documented infection had bacteraemia, with a predominance of single Gram-positive microorganisms. Bacteraemia with Gram-negative microorganisms was a rare event and occurred in only two patients receiving levofloxacin (one patient with a single Gram-negative organism and one with polymicrobial bacteraemia), and was not observed among patients receiving ciprofloxacin-phenethicillin. The numbers of clinically documented infections and of fever of unknown origin were comparable in the two groups.

Table 3. Classification of infections.

	Levofloxacin		**Ciprofloxacin**		Relative risk	
	n	%	n	%	(95%CI)	*P*
Total patients	n = 122		n = 120			
Microbiologically documented infection	21	17.2	22	18.3	0.94 (0.55-1.62)	0.82
Bacteremia	17	13.9	20	16.7	0.84 (0.46-1.52)	0.55
Single Gram-positive isolate	13	10.7	15	12.5	0.85 (0.42-1.72)	0.65
Single Gram-negative isolate	1	0.8	0	0.0		0.32
Polymicrobial or other	3	2.5	5	4.2	0.59 (0.14-2.42)	0.46
Pos. sputum or bronchial lavage cultures	1	0.8	2	1.7	0.49 (0.05-5.36)	0.55
Pos. central venous catheter tip cultures	1	0.8	0	0.0		0.32
Pos. mouth and throat cultures	2	1.6	0	0.0		0.16
Clinically documented infection	12	9.8	18	15.0	0.66 (0.33-1.30)	0.22
Lung	6	4.9	10	8.3	0.59 (0.22-1.57)	0.28
Mouth and throat	3	2.5	3	2.5	0.98 (0.20-4.78)	0.98
Skin	1	0.8	3	2.5	0.33 (0.03-3.11)	0.31
Other	2	1.6	2	1.7	0.98 (0.14-6.87)	0.99
Fever of unknown origin	40	32.8	29	24.2	1.36 (0.90-2.04)	0.13

Microbiological evaluations

Throat surveillance cultures (levofloxacin, $n = 448$; ciprofloxacin-phenethicillin, $n = 485$) yielded predominantly VG streptococci (Table 4). From day 1 to day 4, VG streptococci were found in 111 (91%) of 122 patients in the levofloxacin group, compared with 108 (90%) of 120 patients in the ciprofloxacin-phenethicillin group. During the subsequent study period, the number of patients with throat surveillance cultures that yielded VG streptococci decreased in both groups. However, the colonization rate of VG streptococci was reduced faster and to a larger extent over time in the levofloxacin group compared with the ciprofloxacin-phenethicillin group (chi-square for trend, $P = <0.0001$). From day 1 to day 4, 107 (96%) of 111 VG streptococcal isolates from levofloxacin recipients were susceptible to levofloxacin, two (2%) were resistant and two (2%) were intermediately-susceptible. In contrast, only 25 (23%) of 108 VG streptococcal isolates from ciprofloxacin-phenethicillin recipients were susceptible to ciprofloxacin, 12 (11%) were resistant and 71 (66%) were intermediately-susceptible. VG streptococci resistant to phenethicillin were not isolated from patients receiving ciprofloxacin-phenethicillin prophylaxis, although isolates from 30 patients had intermediate susceptibility.

Anal surveillance cultures (levofloxacin, $n = 469$; ciprofloxacin-phenethicillin, $n = 503$) yielded predominantly *Escherichia coli* and other *Enterobacteriaceae*. Eradication of these microorganisms was highly efficient with both prophylactic regimens. At baseline, two patients in each group had quinolone-resistant *E. coli*. Acquired resistance in *E. coli* during the study period was observed for one patient receiving levofloxacin, and for two patients receiving ciprofloxacin-phenethicillin. Most bacterial isolates from blood cultures and from cultures of other sites were resistant to levofloxacin and ciprofloxacin (Table 4). The number and type of bacteria isolated were similar in both groups, with coagulase-negative staphylococci and enterococci identified most frequently.

Tolerance of study drug and adverse events

The questionnaire concerning daily tolerance of the study medication was completed by 100 patients receiving levofloxacin (response rate 82%) and by 79 patients receiving ciprofloxacin-phenethicillin (response rate 66%). From day 4, the mean tolerance score per day was significantly lower for ciprofloxacin-phenethicillin recipients than for patients receiving levofloxacin ($P < 0.05$), indicating that patients considered the intake of levofloxacin less problematic than that of ciprofloxacin-phenethicillin.

Biochemistry values, expressed as maximum CTCAE toxicity grade of transaminases, creatinine and albumin levels, were similar for the two groups, both at baseline and during the study period. In addition, adverse events were documented at the same frequency in the two treatment groups, of which skin rash was observed most frequently (Table 2).

Table 4. Isolated pathogens and resistance patterns.

	Levofloxacin		Ciprofloxacin	
	No. of Patients with isolate			
	Total	Resistant	Total	Resistant
Surveillance Cultures				
Gram-positive microorganisms				
VG Streptococci, day 1-4	111	2	108	12
VG Streptococci, from day 4	67	17	101	51
Beta haemolytic streptococci, day 1-4	12	0	16	0
Beta haemolytic streptococci, from day 4	2	0	1	0
S. aureus, day 1-4	7	0	6	0
S. aureus, from day day 4	0	0	1	0
Gram-negative microorganisms				
E coli, day 1-4	81	2	79	2
E coli, from day 4	12	3	7	4
Other *Enterobacteriaceae*, day 1-4	40	0	42	0
Other *Enterobacteriaceae*, from day 4	5	0	3	0
Pseudomonas aeruginosa	1	0	2	0
Bacterial isolates				
Blood				
Coagulase-negative staphylococci	18	18	21	21
Enterococci	5	5	6	6
VG Streptococci	0	0	2	2
S. aureus	0	0	1	1
Stenotrophomonas maltophilia	1	1	0	0
Serratia marcescens	1	0	0	0
Sputum or broncho-alveolar lavage fluid				
Stenotrophomonas maltophilia	2	1	0	0
Legionella pneumophila	0	0	1	1
Other sites				
Enterococci	3	2	2	1

Mortality

The overall mortality rate was 2.5% (six of 242 patients). Two patients in the levofloxacin group died, one from sinusoidal obstruction syndrome of the liver, and the other from respiratory failure caused by a pulmonary infection, with no causative microorganism identified. Four patients died in the ciprofloxacin-phenethicillin group, one from a probable

infection with *Aspergillus fumigatus*, a second from cardiac arrest, and two from respiratory failure. Of the last two patients, the alveolar lavage fluid from one patient yielded *Flavobacteria* and *Candida albicans*, while cultures remained negative for the other patient.

Discussion

The results of this randomized controlled clinical trial demonstrate that levofloxacin and ciprofloxacin plus phenethicillin are equally successful as antibacterial prophylaxis for neutropenic patients with haematological malignancies. Failure of prophylaxis, as the primary outcome measure, was observed at the same frequency in the two treatment groups, as were its composites: the initiation of broad-spectrum antibacterial antibiotics, and the need for change of the prophylactic regimen. Other infection-related outcomes, e.g., the time to failure of prophylaxis, occurrence of fever, the number of patients with a microbiologically documented infection, and the number of patients who received broad-spectrum intravenous antibiotics, did not favour either of the prophylactic strategies. However, patients receiving ciprofloxacin-phenethicillin had a discrete, but significantly longer, duration of neutropenia of *c.* 1.6 days. This finding probably accounts for the trend towards a longer duration of hospital stay for these patients, and may be an explanation for the (almost significant) higher number of days for which patients in the ciprofloxacin-phenethicillin group needed intravenous antibiotics. It is well-known that prolonged administration of β-lactam antibiotics may induce neutropenia, probably because of a direct toxic effect on the bone marrow or an immune-mediated effect.[14,15] Considering the fact that duration of neutropenia has been identified as an independent risk-factor for the occurrence and severity of infections, and as a critical factor in a successful outcome, this finding may be of clinical importance.[16,17]

It was assumed that patients receiving one tablet of levofloxacin per day would tolerate the study medication better than patients receiving two tablets of ciprofloxacin plus four tablets of phenethicillin. The results of the questionnaire confirmed this supposition. From day 4, the mean tolerance score for levofloxacin was significantly higher than the score for ciprofloxacin-phenethicillin. In line with these results, a change of prophylaxis because of intolerance of the study drugs was necessary for significantly more patients receiving ciprofloxacin-phenethicillin than for those receiving levofloxacin. Since these patients are commonly suffering from discomforting nausea and mucositis, a better tolerance of prophylactic medication is important and may improve therapy compliance. However, the

results of the questionnaire need to be interpreted with caution. The response rate was 82% in the levofloxacin group and 66% in the ciprofloxacin-phenethicillin group, which may indicate selection bias. Patients were sometimes disappointed not to have been assigned to receive levofloxacin, and other patients became very ill during the study period. It is possible that these patients, in particular, were less motivated or less able to complete and return the questionnaire.

The prophylactic administration of both ciprofloxacin-phenethicillin and levofloxacin resulted in good control over Gram-negative bacteria, and only two patients, both receiving levofloxacin, developed Gram-negative bacteraemia. The efficacy of quinolone prophylaxis in reducing Gram-negative infections has been well-documented, although the emergence of quinolone-resistant bacteria, particularly *E. coli*, has been reported.[18,19] Moreover, prophylaxis with the older-generation quinolones, e.g., ciprofloxacin, has been associated with an increase in the number of Gram-positive infections. Levofloxacin and other newer quinolones have enhanced activity against Gram-positive microorganisms, and may potentially overcome this problem. However, early reports concerning levofloxacin administered as antibacterial prophylaxis suggest that its use may be associated with the selection of quinolone-resistant VG streptococci.[12,13] This is a major drawback, as these microorganisms have been reported to be responsible for up to 39% of cases of bacteraemia in neutropenic patients, and may result in serious complications, including endocarditis, adult respiratory distress syndrome, shock and even death.[4,13,20] In the present study, surveillance cultures yielded levofloxacin-resistant VG streptococci from 17 (14%) of 122 levofloxacin recipients. In these patients the prophylactic regimen was adjusted, in most instances by the addition of penicillin. This proved to be a valuable approach, as no bacteraemia with VG streptococci occurred in the levofloxacin group. In agreement with data published previously, the bacteria isolated most frequently from patients with a bloodstream infection were coagulase-negative staphylococci and enterococci.[20,21] As expected, these bacteria were invariably resistant to levofloxacin and ciprofloxacin. This finding should be taken into account in the choice of empirical antibiotic therapy, and underscores the importance of meticulous care of central venous access devices.

In conclusion, levofloxacin was as efficacious as ciprofloxacin plus phenethicillin for the prevention of bacterial infections in neutropenic patients with cancer. However, levofloxacin is better-tolerated, which may benefit compliance with therapy. Resistance in VG streptococci does occur, but this problem appears to be manageable if resistance patterns are monitored closely. The present study does not answer the question as to which patients with neutropenia benefit most from the prophylactic administration of levofloxacin and other quinolones, nor

does it support the unlimited or uncontrolled use of these agents. As outlined in the guidelines published by the Infectious Diseases Society of America,[22] routine quinolone prophylaxis for all neutropenic patients is not recommended. Based on the estimated infection risk for their own category of neutropenic patients, and with careful consideration of local antimicrobial resistance patterns, physicians should weigh the benefits of quinolone prophylaxis against the potential dangers of this approach.

Acknowledgements

We thank M. Gittelbauer, R. Piqué, F. Tangali, S. Straver, I. Schouten and H. Rouwenhoff from the Department of Medical Microbiology and Infection Control for their work on culture data and susceptibility testing. We also thank M. Leisink, Y. den Hartog, C. van Roessel, C. Eeltink and J. van Wegen from the Haematology Trial Office for their contribution to data management. This investigator-driven study was sponsored, in part, by a grant from Sanofi Aventis, Gouda, The Netherlands.

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Chapter 5

Amphotericin B colloidal dispersion (Amphocil) vs fluconazole for the prevention of fungal infections in neutropenic patients: data of a prematurely stopped clinical trial.

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Bone Marrow Transplantation 2000;25:879-884

Abstract

We conducted an open label, randomized clinical trial to compare amphotericin B colloidal dispersion (ABCD, Amphocil) 2 mg/kg/day intravenously with fluconazole 200 mg/day orally, for the prevention of fungal disease in neutropenic patients with haematological malignancies. In the event of unresolved fever after 4 days of empirical antibacterial therapy, patients in both treatment groups were to receive ABCD, 4 mg/kg/day. However, the study had to be stopped in an early phase, due to severe side-effects of ABCD. A total of 24 patients were enrolled, 12 patients were randomly assigned to receive prophylactic ABCD, which was administered for a mean of 13.9 days. Fluconazole prophylaxis was given to 12 patients for a mean of 21.2 days. Therapeutic ABCD, 4 mg/kg, was initiated in four patients because of suspected fungal infection, all of whom had initially received fluconazole. A high rate of infusion-related toxicity of ABCD was observed. Chills occurred in 15/16 ABCD recipients (94%), accompanied by a temperature rise of ≥2°C in 4/16 patients and of ≥1°C but <2°c in 10/16 patients. Other ABCD-related adverse events were hypotension (4/16), nausea with vomiting (5/16), tachycardia (7/16), headache (3/16) and dyspnoea (3/16). For premedication patients received: antihistamines (12/16), hydrocortisone (9/16) and/or morphine (6/16). ABCD was discontinued in 8/16 patients (50%) due to side-effects, which ultimately dictated early termination of the study. we conclude that ABCD is not suitable for antifungal prophylaxis in neutropenic patients due to severe infusion-related side-effects. Subject numbers were too low for conclusions on variables of antifungal efficacy.

Introduction

Superficial or invasive fungal infections, most often caused by *Candida* and *Aspergillus* species, are among the most serious complications after chemotherapeutic treatment of haematological malignancies.[1-3] In view of the poor prognosis of disseminated fungal disease, many centres administer antimycotic agents prophylactically. Fluconazole has emerged as the most widely used prophylactic agent in neutropenic patients and has been used in doses varying from 50 to 400 mg.[4-10] Its optimal dose in antifungal prophylaxis remains to be determined.[5,6] Not only has fluconazole been found to be highly effective in eliminating colonization and infection by *Candida* spp. in patients with leukaemia,[11] but interestingly, the drug has also been proven to significantly reduce the incidence of, and mortality from systemic fungal infections in allogeneic stem cell transplantation recipients.[4,9] An important limitation to the use of fluconazole however, is its lack of efficacy against *Aspergillus* spp. Moreover, some *Candida* spp. other than albicans, including *C. glabrata* and *C. krusei* are less sensitive or resistant.

Amphotericin B (AmB) remains the gold standard of antifungal therapy and is attractive as a prophylactic agent because of its broad coverage of most fungal organisms. However, its use is seriously hampered by dose-limiting toxicity, with acute infusion-related toxicity and renal failure being the most important adverse effects. To circumvent toxicity, low doses of intravenous AmB have been used in antifungal prophylaxis with ambiguous results.[12-14] Lipid formulations of AmB have been designed to improve efficacy and to reduce toxicity of the parent compound, thereby allowing the possibility of administering higher doses of AmB. Amphotericin B colloidal dispersion (ABCD, Amphocil; Sequus Pharmaceuticals, Brentford, UK) is a colloidal formulation of amphotericin B complexed to cholesteryl sulphate in a 1:1 ratio, forming disc-shaped particles. In various open-label clinical trials, the use of ABCD showed promising results with regard to its antifungal efficacy and its safety.[15-19] Its assumed reduced toxicity offers the opportunity of investigating the feasibility of AmB as antifungal prophylaxis. In a recent randomized trial we reported a 100% mortality in patients with proven fungal infections due to *Aspergillus* or *Mucor* spp., the overall incidence of such infections in our population being 5%.[10] In an attempt to reduce both incidence of, and mortality from these infections, an open-label, randomized clinical trial was initiated, to compare the safety and efficacy of intravenous ABCD with oral fluconazole in the prevention of fungal disease in neutropenic patients with haematological malignancies. For the prophylactic use of ABCD we decided to administer half of the daily dose that is generally

recommended for antifungal therapy. Unfortunately, due to excessive toxicity of ABCD the study had to be stopped in an early phase, after the randomization of 24 patients.

Patients and methods

Patients

Consecutive patients, aged 18-75 years, who were to receive treatment for haematological malignancies with or without peripheral blood stem cell transplantation were enrolled. An anticipated granulocytopenic period of at least 10 days was required. Patients were excluded if they had a previous history of allergy or hypersensitivity to any lipid preparation of AmB, or to other polyene or azole antimycotic agents. Patients were considered not eligible if they had overt infection requiring treatment at entry, if they had received treatment with systemic or topical antifungal drugs within 2 weeks prior to enrolment, or if there was hepatic or renal impairment, defined as elevation of liver enzymes more than three times the upper limit of normal, or a creatinine clearance of less than 15 ml/min, respectively. The study was approved by the institutional ethical and scientific committees. Written informed consent was obtained from all patients.

Study protocol

The study was designed as a randomized, open-label clinical trial. Patients were randomly assigned to receive once-daily prophylaxis with either fluconazole 200 mg orally, or ABCD 2 mg/kg i.v. ABCD was prepared as recommended by the manufacturer and was administered at an infusion rate of 1 mg/kg/h. No other drugs were co-administered during ABCD infusion. Premedications were not given before the first dose of ABCD. In case of infusion-related toxicity, clemastin, hydrocortisone or morphine were initiated sequentially or in combination for premedication, depending on the severity of symptoms. Study treatment was started on the first day of the chemotherapeutic regimen and was continued until neutrophil recovery (ANC $>0.5 \times 10^9$/l) or failure of prophylaxis, defined as the necessity of initiating therapeutic ABCD, treatment interruption due to side-effects or mortality. For prevention purposes all patients received ciprofloxacin, 500 mg twice daily and azithromycin 250 mg once daily. Nasal AmB was given, 2 mg three times a day, into both nasal orifices.

Daily clinical assessments were performed, including documentation of signs and symptoms of infection and of tolerance to the study medication. The highest grade of infusion-related

toxicity that occurred during the study period was recorded. Routine haematology and clinical chemistry tests were obtained three times weekly. Surveillance cultures for fungal and bacterial organisms were taken from throat and anus prior to the first dose of the study drug and, thereafter, once weekly.

When axillary temperature increased above 38.5°C, clinical evaluation was performed and empirical antibiotic therapy was initiated, as described in detail earlier.[10] Empirical treatment consisted of cefpirome 2000 mg, twice daily i.v. If subsequently, fever did not resolve within 96 h, patients in both treatment groups were to receive ABCD, 4 mg/kg once daily i.v.

Definition of infection

Fungal colonization was considered to be present if one or more surveillance culture yielded a fungus, in the absence of any clinical symptoms or signs of infection. Suspected systemic fungal infection was defined as fever, persisting after 96 h of appropriate antibacterial treatment, necessitating the use of therapeutic ABCD. Proven fungal infection was defined as a positive culture or characteristic histopathological findings on tissue biopsy specimens obtained from a normally sterile site.

Statistical analysis

Results from all patients were analysed according to the intention-to-treat principle. Categorical data were analysed by Fisher's exact test, Student's *t*-test was used for comparison of means. All tests were two-sided and in all cases a P value less than 0.05 was considered statistically significant. To detect significant differences between groups in the number of proven and suspected fungal infections, it was calculated that the study should include a total of 220 patients.

Results

Before the early termination of the study, 24 patients with haemato-oncological diseases were included, 12 receiving ABCD and 12 fluconazole. Patient characteristics are given in Table 1. The two groups were comparable for sex, type of diagnosis, number of stem-cell transplants and duration of neutropenia. The ABCD group contained two patients receiving allogeneic or syngeneic stem cell transplantation. Patients in the fluconazole group were significantly older than patients in the ABCD group (P <0.006).

Table 1. Base-line characteristics of the study patients.

Characteristics	Medication			
	Fluconazole		**ABCD**	
	n	*(%)*	*n*	*(%)*
Total number of patients	12	(100)	12	(100)
Sex				
Male	6	(50)	8	(67)
Female	6	(50)	4	(33)
Age				
Mean age (yrs)[a] ± SD	55.5 ± 7.6		45.0 ± 9.2	
Range (yrs)	39-65		32-60	
Diagnosis				
AML	3	(25)	3	(25)
ALL	0	(0)	2	(17)
CML	0	(0)	1	(8)
Lymphoma	5	(42)	1	(8)
Aplastic anaemia	1	(8)	0	(0)
Multiple myeloma	3	(25)	5	(42)
Stem cell transplantation				
Autologous	8	(67)	7	(58)
Allogeneic	0	(0)	1	(8)
Syngeneic	0	(0)	1	(8)
Patient receiving GCSF	3	(25)	3	(25)
Duration of neutropenia[b]				
Mean (days) ± s.d.	23.8 ± 7.3		21.6 ±7.1	
Range (days)	6-29		6-29	

a $P < 0.006$

b from start of chemotherapy until ANC >0.5 x 10^9/L

Study drug administration

Prophylactic ABCD, 2 mg/kg, was administered to 12 patients for a mean of 13.9 days (range 1-27). Fluconazole prophylaxis, 200 mg/day, was given to 12 patients for a mean of 21.2 days (range 13-35). The mean number of days of prophylactic treatment differed significantly between the two groups (P = 0.033), due to withdrawals in the ABCD group. ABCD, 4 mg/kg, was initiated for suspected fungal infection in four patients, all initially receiving fluconazole prophylaxis. ABCD was administered to these patients for a mean of 2.3 days (range 1-3).

Blood chemistry

There were no clinically significant changes in haematology or clinical chemistry parameters in either group of patients as compared with baseline values. In particular, no renal toxicity

was observed. In one patient receiving ABCD prophylaxis, bilirubin values increased to five times the upper limit of normal, while aminotransferases remained within the normal range. Although these abnormalities were most likely associated with veno-occlusive disease, a relation to the study-drug could not be ruled out and the patient was withdrawn from the study.

Adverse events

No patient in the fluconazole group experienced side-effects which were deemed related to the study drug. A total of 176 administrations of ABCD administered for either prophylactic (167) or therapeutic (9) reasons in 16 patients was documented. A high rate of adverse events was observed in the ABCD group, which were mainly infusion-related (Table 2). Moderate to severe chills were observed in 15 (94%) of 16 patients receiving ABCD, mostly occurring within 30 min after the start of the infusion. Chills were accompanied by a temperature rise of 2°C in 4/16 patients and of 1-2°C in 10/16 patients. The mean maximal daily temperature during the first 3 days of patients receiving prophylactic study-treatment was significantly higher in the ABCD group vs the fluconazole group (Figure 1). Intravenous administration of morphine proved to be an effective strategy for the acute termination of rigors and chills, and had to be used for this reason in nine patients receiving the first dose of either prophylactic or therapeutic ABCD (Table 3).

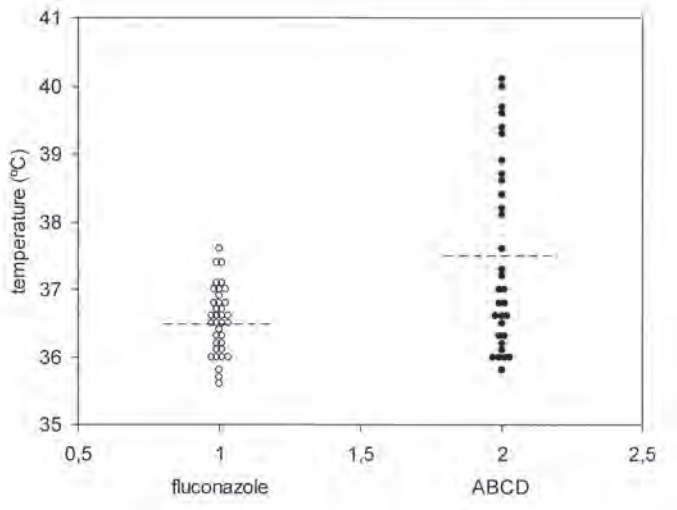

Figure 1 Highest daily body-temperature during the first three days on prophylactic treatment: ABCD recipients (•), mean: 37.5 °C ± 1.38 and fluconazole recipients (o), mean 36.5 °C ± 0.49, $P = 0.001$.

In three patients pulmonary symptoms were observed. One patient experienced a tolerable but persisting shortness of breath during consecutive administrations of prophylactic ABCD. The other two patients, after receiving the first and second dose of therapeutic ABCD respectively, developed dyspnoea, accompanied by bronchospasm, reduced oxygen saturation and in one, marked cyanosis. ABCD was discontinued permanently and both patients were subsequently treated with steroids, oxygen, sympathomimetics and diuretics. A chest X-ray in one of these patients revealed diffuse interstitial oedema, which resolved completely over the ensuing days.

Table 2. Infusion-related reactions to Amphotericin B colloidal dispersion at the 2 mg/kg and 4 mg/kg dose.

Adverse events	ABCD dose				Total	
	2 mg/ kg		4 mg/ kg			
	n	(%)	n	(%)	n	(%)
Total number of patients	12	(100)	4	(100)	16	(100)
Infusion related chills	11	(92)	4	(100)	15	(94)
Mild-moderate	6	(50)	2	(50)	8	(50)
Severe, despite treatment	5	(42)	2	(50)	7	(44)
Infusion related fever						
Rise < 1°C	1	(8)	0	(0)	1	(6)
Rise 1-2 °C	6	(50)	4	(100)	10	(63)
Rise ≥2°C	4	(33)	0	(0)	4	(25)
Hypotension						
Systolic BP ≤ 90 mmHg	2	(17)	2	(50)	4	(25)
Nausea	1	(8)	1	(25)	2	(13)
Nausea, with vomiting	5	(42)	0	(0)	5	(31)
Tachycardia	4	(33)	3	(75)	7	(44)
Headache	3	(25)	0	(0)	3	(19)
Dyspnoea	1	(8)	2	(50)	3	(19)

Withdrawals

Two of 15 patients experiencing infusion-related toxicity were withdrawn after the administration of the first dose of ABCD. The remaining 13 patients all received premedications prior to subsequent ABCD infusions (Table 3). These medications were used both as sequential single drug therapy and in combination. After the initiation of premedications only one patient reported complete symptomatic relief, seven patients experienced ongoing but tolerable side-effects during the study period. Symptoms in this group varied from a generalized cold feeling during ABCD infusion to mild chills. Nevertheless, one of these patients had to be withdrawn, because of elevation of liver

enzymes, as outlined above. Severe and persisting infusion-related toxicity, which occurred despite premedication, necessitated discontinuation of the study-drug in five additional patients, which ultimately resulted in a total number of eight subjects withdrawn. Two of these patients were receiving ABCD for a suspected fungal infection, necessitating the continuation of antifungal therapy. For that reason ABCD was replaced by conventional AmB, 0.7 mg/kg i.v. Remarkably, during treatment with conventional AmB, combined with the same premedication as used while administering ABCD, all side-effects subsided, allowing continuation of antifungal therapy until neutrophil recovery.

Table 3. Medications used for the termination of ABCD related adverse events and subsequent medications

Medications	ABCD dose				Total	
	2 mg/ kg		4 mg/ kg			
	n	(%)	*n*	(%)	*n*	(%)
Morphine at the first episode of chills	6	(50)	3	(75)	9	(56)
Premedications						
Antihistamines	9	(75)	3	(75)	12	(75)
Hydrocortisone	6	(50)	3	(75)	9	(56)
Morphine	3	(25)	3	(75)	6	(38)

Fungal infections

There were too few patients completing the study to allow meaningful comparison of the number of fungal infections and colonization data. No proven systemic fungal infections occurred in either of the treatment groups. Clinically apparent or culture documented superficial fungal infections were not observed. Suspected fungal infections occurred in four patients in the fluconazole group. Subsequently, these patients received ABCD, 4 mg/kg i.v. One patient initially receiving ABCD prophylaxis was suspected of having a fungal infection 2 weeks after she had been withdrawn from the study because of side-effects of ABCD.

Mortality

Overall two (17%) patients in the group receiving prophylactic ABCD died and no deaths occurred in the group receiving fluconazole. In neither of these deaths did a relation with the study-drug seem likely; one patient in the ABCD group died from diffuse pulmonary haemorrhage during treatment with L-asparaginase. The other patient died from respiratory failure, 3 weeks after withdrawal.

Termination of the study

A variety of strategies not integral to the protocol was used to cope with the high rate of infusion-related toxicity. Three different batches of ABCD were used to rule out quality differences of the compound. In individual cases the infusion time was sometimes prolonged for up to 4 h. However, ultimately all measures failed to increase tolerability and the study was prematurely closed, according to GCP guidelines.

Discussion

Although the use of antifungal prophylaxis in neutropenic patients, especially in low-risk autotransplant recipients, is certainly debatable, mortality from these infections remains high. Lipid formulations of AmB appear attractive as antifungal prophylaxis because these compounds are claimed to decrease toxicity, while maintaining or even enhancing therapeutic efficacy. In two randomized, placebo-controlled clinical trials, prophylactic administration of AmBisome to patients with haematological malignancies was associated with a statistically significant reduction of fungal colonization.[20,21] Both studies, however, failed to show a significant reduction in proven fungal infections, possibly because patient samples were too small. Our randomized, open label clinical trial was designed to compare the safety and efficacy of ABCD with fluconazole in the prevention of fungal infections in neutropenic patients with haematological malignancies. However, severe and unexpected toxicity of ABCD resulted in the early withdrawal of eight patients, comprising 50% of the ABCD recipients. In all instances there was a direct and obvious correlation with the infusion of ABCD. Infusion-related chills were observed in 94% of the patients receiving ABCD, necessitating extensive use of premedications. Three patients had pulmonary symptoms, with marked dyspnoea bronchospasm and hypoxaemia in two. Acute pulmonary toxicity is a known complication of AmB infusions and has been reported in association with its lipid formulations before, including ABCD.[22-24]

We did not anticipate the occurrence of adverse events to such a large extent at the 2 mg/kg dose level. In early open-label phase I/II trials, escalating ABCD doses of up to 6 mg/kg/day were administered.[15,16] Although the frequency of infusion-related toxicity was considerable, occurring in about 65% of patients, the rate and severity of adverse events increased substantially in patients receiving >4 mg/kg/day compared with those receiving 3-4 mg/kg/day. The maximum tolerated dose was set at 7.5 mg/kg/day. Infusion-related events

were reported to be well controllable or to disappear completely, once premedication was added. Moreover, during the course of the treatment period the proportion of patients experiencing infusion-related events tended to diminish steadily.[15,16,18] These findings are in sharp contrast with our results. One explanation could be that both patients and treating physicians are less willing to accept moderate to severe adverse effects when administering ABCD for prophylactic reasons. However, in the present study, ABCD related toxicity was also considered intolerable in patients with suspected fungal infections, receiving a therapeutic dose of ABCD at 4 mg/kg/day. After being withdrawn from the study two of these patients received conventional AmB without appreciable toxicity. This observation is in line with data from a recent randomized double blind study, comparing ABCD (4 mg/kg/day) with conventional AmB (0.8 mg/kg/day) for the empirical treatment of fever in neutropenic patients.[24] The authors report that chills occurred more frequently in ABCD recipients than in patients receiving conventional AmB, 80% vs 65% respectively, resulting in a more frequent use of premedication by ABCD recipients.

We have no proper explanation for the high rate of infusion-related toxicity associated with ABCD, as observed in our patients. The three commercially available lipid formulations (Amphocil, AmBisome and Abelcet) seem to differ substantially with regard to their infusion-related toxicity, probably due to major differences in molecular structure.[25] In general, the rate of acute reactions seems to be minimal for AmBisome and highest for ABCD.[25,26] Severe and unexpected toxicity has been reported with the use of Abelcet recently.[27] The characteristics of the adverse events are very much like the well-known toxic effects of conventional AmB, which have been shown to be associated with increasing plasma levels of TNF-α, IL-1 and IL-6.[28,29]

In earlier reports, the use of ABCD is invariably labelled as 'safe'.[15-19] Although this may hold true with regard to its renal sparing effects, our data indicate, in line with recent observations,[24] that infusion-related toxicity of ABCD must not be underestimated and poses a major drawback to its use. We conclude that ABCD, administered for prophylactic reasons to patients without life-threatening fungal infections, was associated with major and intolerable side-effects during infusion of the compound. Therefore, ABCD appears to be unsuitable for antifungal prophylaxis in neutropenic patients.

Acknowledgements

This study was supported by a grant from Zeneca Farma, Ridderkerk, The Netherlands, distributor of Amphocil for Europe.

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Chapter 6

Effects of cyclosporin A on single-dose pharmacokinetics of intravenous itraconazole in patients with haematological malignancies.

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Therapeutic Drug Monitoring, accepted for publication

Abstract

An open-label, clinical pilot study was performed to study the effect of cyclosporin A (CsA) on single-dose pharmacokinetics of itraconazole in patients with a haematological malignancy. Patients (n=10), admitted for an allogeneic stem-cell transplantation, received a single dose of 200 mg itraconazole, in an one hour intravenous infusion, during their treatment period before initiation of CsA. This was repeated during the period that CsA was administered and a steady state concentration of CsA was achieved (trough whole blood level 200-400 ng/ml). After both administrations of itraconazole, serum pharmacokinetics of itraconazole and hydroxy (OH) itraconazole were determined during 24 hours. The results were compared, with each patient acting as his/her own control. Exposure to itraconazole, as measured by the $AUC_{[0\text{-}24h]}$, was not significantly altered when combined with CsA. Large inter-individual variations were observed in AUC values among patients. In contrast, exposure to OH-itraconazole was significantly increased when itraconazole was co-administered with CsA (median increase of $AUC_{[0\text{-}24h]}$ 49%), with significant prolongation of T_{max} and $T_{1/2}$ (median increase of T_{max} 37% and $T_{1/2}$ 176%). These differences may be due to variability in affinity of itraconazole, OH-itraconazole and CsA for the cytochrome P450 (CYP) 3A4 metabolic system and the occurrence of P-gp polymorphisms. In conclusion, exposure to OH-itraconazole, but not to itraconazole is increased when itraconazole is co-administered with CsA. Though the interaction profile of itraconazole and CsA remains complex, these findings may be of importance in patients, in whom monitoring of itraconazole serum levels is warranted, for example in those with life-threatening fungal infections, or in those who receive concurrent CYP inducers or inhibitors.

Introduction

Itraconazole is a broad-spectrum triazole antifungal agent, that has proven to be effective in the prevention and treatment of systemic fungal infections in patients with neutropenia.[1-3] The antifungal activity from itraconazole originates from inhibition of the fungal cytochrome P450 (CYP) 3A iso-enzyme, which plays a central role in the synthesis of ergosterol, a vital component of the fungal cell membrane. Although with much lower affinity, itraconazole and its main metabolite, hydroxy-itraconazole (OH-itraconazole) are also inhibitors of this enzyme in humans.[4-6] Because the CYP3A isoenzymes are involved in the metabolic pathway of many drugs, itraconazole and OH-itraconazole have the potential to modify the pharmacokinetics of these medications. In addition, itraconazole is both inhibitor and substrate of the ATP-dependent cell membrane transporter system P-glycoprotein (P-gp, multidrug resistance 1 [MDR1]), which is involved in the clearance of many drugs.[7,8] Polymorphisms of the MDR1 gene have been reported to be associated with alterations in disposition kinetics and interaction profiles of clinically useful drugs, including digoxin, tacrolimus and cyclosporin A, though data on itraconazole are lacking.[9]

One of the most important drug interactions in which itraconazole is involved, is that with cyclosporin A (CsA). CsA is a calcineurin inhibitor, frequently used for the prevention of graft-versus-host disease in patients undergoing allogeneic stem cell transplantation. Like itraconazole, CsA is both substrate and inhibitor of CYP3A4 and P-gp and it has been well documented that the combination of these two drugs results in increased CsA serum concentrations.[10-15] This may have important toxic effects, especially for kidney function.

Though it is well documented that administration of itraconazole increases CsA serum concentrations, there are no data on the effects of CsA on itraconazole pharmacokinetics. Monitoring of itraconazole serum concentrations may be warranted in patients who use other drugs that interact with CYP3A4, and when target drug concentrations are to be achieved for optimal antifungal prophylaxis or therapy.[16,17] Moreover, patients receiving CsA are systematically excluded from studies investigating itraconazole pharmacokinetic parameters, and the question remains as to whether that is justified. We therefore conducted an open-label, pilot study in haematological patients receiving allogeneic stem-cell transplantation, to study the effect of CsA on single-dose pharmacokinetic parameters of itraconazole. In addition, the P-gp genotype was determined, to study the possible effects of single nucleotide polymorphisms (SNP) in the MDR1 gene on itraconazole pharmacokinetics.

Materials and methods

Patients

Patients aged 18-75 years, admitted to the department of Haematology of the VU University Medical Center, Amsterdam, The Netherlands, and scheduled to receive allogeneic stem cell transplantation for the treatment of haematological malignancies, were eligible. Reasons for exclusion were a history of previous allergy or known hypersensitivity to itraconazole, known hepatic impairment as determined by elevation of any liver function test greater than three times the upper limit of normal, or a creatinine clearance under 15 ml/min. Patients with overt infection at baseline were not eligible. Moreover, patients who used other drugs known to affect CYP and P-gp function, for example statins, were excluded.

Due to ethical considerations the number of patients in this pilot study was limited to 10. The protocol was approved by the institutional scientific and ethical committees and all participating patients provided written informed consent. The study was performed according to the recommendations of the Helsinki Declaration.

Study design

The study was designed as a single center, open-label, prospective pharmacokinetic trial and comprised two phases, with patients acting as their own controls. Phase 1 was scheduled before initiation of CsA and phase 2 as soon as a steady state concentration of CsA was achieved within the therapeutic range, defined as a trough whole blood level of 200-400 ng/ml. In both phases patients received a single dose of 200 mg itraconazole (Trisporal, for *iv* administration (10 mg/ml) Ortho-Biotech, Tilburg, the Netherlands). Itraconazole was prepared as recommend by the manufacturer, adding 25 ml (10 mg/ml) itraconazole solution to 50 ml NaCl 0.9%. Of the dilution obtained 60 ml (200 mg) was administered by an one hour infusion. Subsequently, serial blood samples were obtained at 1 hour (end of the infusion);1.25; 1.5; 2; 3; 5; 7; 9; 12; 16 and 24 hours after start of the infusion. Samples were collected in 10 ml coagulation tubes, via a central venous catheter. Serum was separated from the clot by centrifugation (3000 rpm for 10 minutes) and subsequently stored at -40°C until analysis. In order to elucidate the possible effects of the P-gp genotype on pharmacokinetic parameters, an extra 5 ml EDTA blood sample was drawn at study entry, samples were stored at -40°C until analysis.

Sample analysis

Itraconazole and its metabolite OH-itraconazole were both quantitatively determined with a validated reversed-phase high-performance liquid chromatographic assay (RP-HPLC) with ultraviolet detection at a wavelength of 258 nm, as described previously.[18] The analysis took place at ambient temperature. The assay was linear up to 3.2 mg/L and the lower limit of quantification was 0.03 mg/L, for both itraconazole and OH-itraconazole. CsA blood concentrations were measured with a fluorescence polarization immunoassay (FPIA) for the TDx (Abbott Laboratories, Chicago, USA), following the instructions of the manufacturer.

P glycoprotein pharmacogenetic analysis

For determination of the P-gp genotype, 5 ml EDTA blood was taken. Genomic DNA was isolated and PCR amplifications were performed by standard techniques. The three most important single nucleotide polymorphisms (SNP) in the MDR1 gene were determined, as described previously.[19,20]

Pharmacokinetic analysis

Pharmacokinetic parameters were first derived by use of noncompartmental methods in WinNonlin (Version 1.5, Scientific Consulting, Inc). The highest observed serum concentration was defined as the C_{max}, with the corresponding sampling time as T_{max}. The area under the serum itraconazole and OH-itraconazole concentration versus time curve from 0-24 h ($AUC_{[0\text{-}24h]}$) was obtained by use of the linear trapezoidal rule. The concentration at 24 hours after the infusion of itraconazole was defined as the trough concentration (C_{min}). The terminal log-linear period (log C versus T) was defined by the last data points ($n \geq 3$) by visual inspection. The absolute value of this slope (λz) was calculated by least squares regression analysis. The elimination half-life ($T_{1/2}$) was calculated using $T_{1/2}=\ln 2/\lambda z$. The clearance was calculated by dividing the dose by the AUC extrapolated to infinity (AUC_{inf}). The volume of distribution in steady-state (V_{ss}) was estimated from the mean residence time extrapolated to infinity, times clearance.

Statistical analysis

Statistical calculations were performed with the Statistical Product and Service Solutions (SPSS) for Windows, version 9.0. To detect a difference in the pharmacokinetic parameters of itraconazole and OH-itraconazole alone (phase 1) and itraconazole and OH-itraconazole in

combination with CsA (phase 2), the Wilcoxon's Matched-Pairs Signed-Ranks Test was used. A p-value of 0.05 or less was considered to indicate a statistically significant difference.

Results

Patients

Eleven patients were enrolled in this study, and 10 successfully completed both phases. One patient was excluded from analysis, because treatment with itraconazole was clinically indicated, which precluded a single-dose study of phase 2. The baseline characteristics of the remaining 10 evaluable patients are shown in Table 1. Itraconazole was well tolerated, both as single drug infusion (phase 1), as well as in combination with CsA (phase 2), and no adverse effects occurred. No significant changes in ASAT, ALAT, bilirubin, alkaline phosphatase or serum creatinine were observed.

Table 1. General characteristics

Characteristic	Number of patients (n)
Number of patients	10
Age (years, mean ± SD)	48.2 ± 11.7
Bodyweight (kg, mean ± SD)	83 ± 15.3
Creatinine (μmol/L, mean ± SD)	89.7 ± 11.9
Underlying disease	
Acute Myeloid Leukaemia	1
Acute Lymphoblastic Leukaemia	2
Chronic Myeloid Leukaemia	1
Multiple myeloma	3
Non Hodgkin's lymphoma	3
Conditioning regimen	
Busulfan/ cyclophosphamide	1
Cyclophosphamide/ TBI[a]	2
Fludarabin	7

a. TBI; Total Body Irradiation

Pharmacokinetic analysis

The median serum concentration of itraconazole versus time curve, determined on the pharmacokinetic study days, are shown in Figure 1. Median values and interquartile ranges (IQR) of the serum pharmacokinetic parameters of itraconazole alone and in combination

with CsA are shown in Table 2. The effects of co-administration of CsA on itraconazole pharmacokinetics appeared to be highly unpredictable. There was a median increase of $AUC_{[0\text{-}24hr]}$ of 39% (IQR -43 - 215%). However, this increase was not statistically significant (p=0.6) and the negative 25% percentile (-43%) and positive high 75% percentile (215%) reflect the large inter-individual variation in change of AUC among the 10 patients, in phase 1 and phase 2. In addition, no statistically significant differences were found between phase 1 and 2 among other pharmacokinetic parameters, including C_{max}, T_{max}, $T_{1/2}$, clearance and V_{ss}. The median serum concentration of OH-itraconazole versus time curve is shown in Figure 2. The corresponding pharmacokinetic parameters of OH-itraconazole alone and in combination with CsA are shown in Table 3. The exposure to OH-itraconazole, as measured by the $AUC_{[0\text{-}24h]}$ was significantly increased during the phase that CsA was administered as compared with phase 1 (p=0.0039). The median increase of $AUC_{[0\text{-}24h]}$ for OH-itraconazole was 49% (IQR 24-95%). T_{max} and $T_{1/2}$ both were significantly prolonged (p=0.0117 and p=0.0039, respectively) with a median increase of T_{max} of 37% (IQR 12-118%) and a median increase of $T_{1/2}$ of 176% (IQR 130-291). This was reflected by a decreased clearance (p=0.0039) with a median decrease of 63% (IQR 46 - 76%).

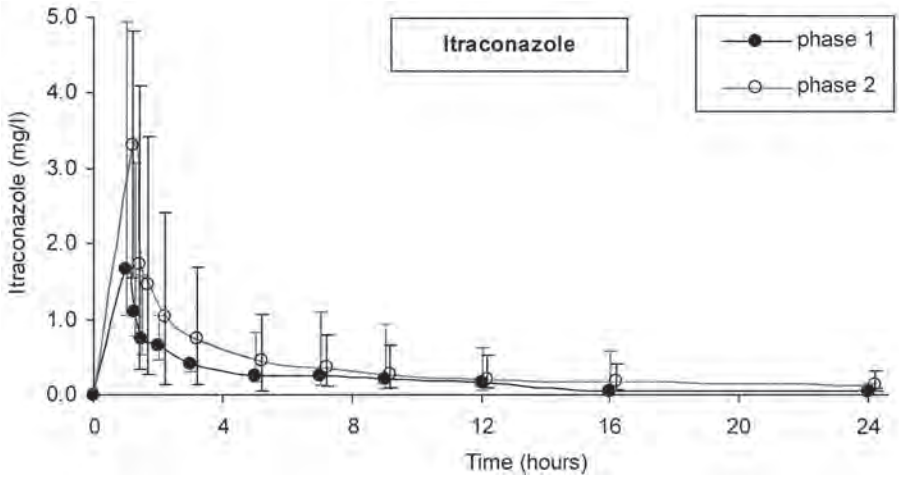

Figure 1. Median itraconazole serum concentration versus time curve, phase 1 itraconazole alone, phase 2 with cyclosporin A. The error bars represent the 25% and 75% percentile.

Table 2. Median pharmacokinetic parameters of itraconazole, alone (phase 1) and in combination with cyclosporin A (phase 2) in 10 patients. The interquartile ranges are represented in parentheses.

Parameter	Phase I		Phase II		
	median	Interquartile range	median	Interquartile range	p-value
$AUC_{(0-24)}$ (h*mg/L)	5.4	(3.0 - 16.2)	8.5	(6.8 - 10.4)	0.6250
C_{max} (mg/L)	1.4	(1.0 - 6.2)	4.0	(2.2 - 5.0)	0.5566
T_{max} (h)	1.0	(1.0 - 1.3)	1.2	(1.1 - 1.4)	0.3223
$T_{1/2}$ (h)	7.1	(3.3 -10.0)	8.6	(5.0 - 13.0)	0.4316
Clearance (L/h)	25.8	(12.7 - 50.4)	15.1	(12.6 - 24.0)	0.3223
Vss calculated (L)	305.9	(67.1 - 442.2)	185.7	(156.4 - 278.0)	0.3223

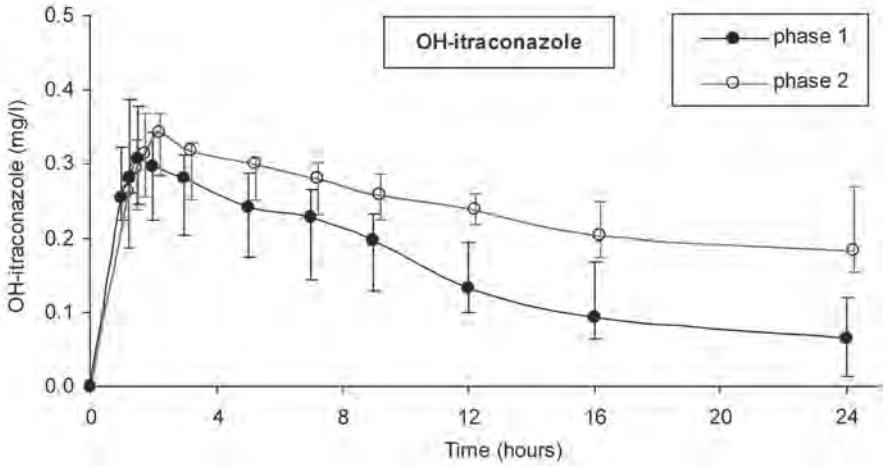

Figure 2. Median OH-itraconazole serum concentration versus time curve, phase 1 itraconazole alone, phase 2 with cyclosporin A. The error bars represent the 25% and 75% percentile.

Table 3. Median pharmacokinetic parameters of hydroxy-itraconazole, alone (phase 1) and in combination with cyclosporin A (phase 2) in 10 patients. The interquartile ranges are represented in parentheses.

Parameter	Phase I		Phase II		
	median	Interquartile range	median	Interquartile range	p-value
$AUC_{(0-24)}$ (h*mg/L)	3.6	(2.6 - 5.0)	5.9	(4.9 - 6.2)	0.0039
C_{max} (mg/L)	0.3	(0.3 - 0.4)	0.3	(0.3 - 0.4)	0.4258
T_{max} (h)	1.3	(1.3 - 1.6)	2.1	(1.5 - 2.5)	0.0117
$T_{1/2}$ (h)	7.8	(5.7 - 13.6)	28.3	(17.6 - 43.5)	0.0039
Clearance (L/h)	50.4	(25.9 - 64.3)	14.6	(9.1 - 19.6)	0.0039
Vss calculated (L)	569.7	(497.0 - 881.5)	681.9	(527.5 - 709.8)	1.0000

P glycoprotein genotype

Results of P-gp genotyping are given in Table 4. Large heterogeneity of the MDR1 gene was observed. However, no clear association was found between polymorphisms in the MDR1 gene and pharmacokinetic parameters of itraconazole and OH-itraconazole.

Table 4. P glycoprotein genotype, reported for three common single nucleotide polymorphisms.

Patient nr.	C1236T	G2677T	C3435T
1	M	M	M
2	H	H	M
3	H	H	H
4	M	M	M
5	H	H	H
6	W	W	W
7	ND	ND	H
8	H	H	H
9	H	H	M
10	H	H	H

W = Wild type, H = Heterozygous, M = Mutant, ND = no detectable PCR product

Discussion

This study was performed to investigate the effect of CsA on pharmacokinetic parameters of itraconazole. The results demonstrate that the exposure to itraconazole, as measured by the $AUC_{[0\text{-}24h]}$, was not significantly altered when combined with CsA. Large inter-individual variations, however, were observed in AUC values among the 10 patients. In contrast, exposure to OH-itraconazole was significantly increased when co-administered with CsA (median increase of $AUC_{[0\text{-}24h]}$ 49%), with significant prolongation of T_{max} and $T_{1/2}$ (median increase of T_{max} 37% and median increase of $T_{1/2}$ 176%).

The explanation for these findings and the relatively unaltered itraconazole levels, as compared with elevated OH-itraconazole levels after co-administration of CsA, is not straightforward. Itraconazole and OH-itraconazole pharmacokinetics are the resultant of many factors and metabolic processes that co-exist, and also may affect each other. For example, in addition to OH-itraconazole, more than 30 metabolites of itraconazole have been identified. In a recent study, using human liver microsomes, it was shown that itraconazole and its sequential metabolites OH-itraconazole, keto-itraconazole and *N*-desalkyl-itraconazole were

all high-affinity ligands of CYP3A4. In fact, these metabolites appeared to be as potent as or more potent CYP3A4 inhibitors than itraconazole itself, and thus may contribute to the inhibition of CYP3A4 in vivo after itraconazole therapy. In addition, itraconazole, OH-itraconazole and CsA are not only substrates and inhibitors of the CYP3A4 system, but also of P-glycoprotein (P-gp).[5,7] Since itraconazole, OH-itraconazole and CsA all interact with CYP3A4 and P-gp, it is difficult to discern their separate effects and dominant avenue of interaction. Large heterogeneity was found in P-gp genotype in our population of patients. P-gp genetic polymorphisms may have an effect on disposition of and exposure to itraconazole, so we hypothesize that this finding may be, at least partially, associated with the pronounced inter-patient variability of itraconazole concentrations in our patient group. Another explanation of the large variability of exposure to itraconazole may be the occurrence of major differences in protein and tissue binding among individual patients. It has been reported that plasma levels of itraconazole at the same daily dose may differ up to 15-fold.[16,17,21] The explanation of the difference between the unaltered exposure to itraconazole when CsA was co-administered, as compared with increased OH-itraconazole levels, can only be speculative. In theory, drugs that require metabolism by the same CYP enzymes compete for binding to- and metabolism by CYP, and may therefore interact. The clinical significance of this interaction will depend on the drugs' relative affinities for binding to these enzymes. *In vitro* studies showed that itraconazole and OH-itraconazole were both high affinity ligands to CYP3A4. However, itraconazole has higher affinity to CYP3A4 than OH-itraconazole, with Michaelis-menten constants (Km), of 3.9 nM and 27 nM respectively.[5] This finding fits our results, due to the higher affinity of itraconazole for binding to CYP, as compared with that of OH-itraconazole or CsA, the elimination of itraconazole may prevail and continue, despite the presence of other competitive substrates, including OH-itraconazole and CsA.

The active metabolite of itraconazole, OH-itraconazole, reaches concentrations in plasma approximately twice those achieved by the parent drug.[6,22] In our study, OH-itraconazole concentrations were comparable to or even lower than itraconazole concentrations. There may be several explanations for this finding. First, itraconazole is subject to considerable first pass metabolism after oral administration. Plasma levels of the first pass metabolite OH-itraconazole may be higher if itraconazole is administered orally, as compared with intravenous administration. In a pharmacokinetic study on switching itraconazole from oral to i.v. use, itraconazole levels continued to rise with a daily dose of 200 mg i.v., while the rate of rise of OH-itraconazole levels slowed, presumably because the rate of exposure to hepatic hydroxylation was reduced.[23] Second, studies in normal volunteers have shown that OH-

itraconazole accumulates at approximately twice the rate of the parent drug.[24,25] Thus, the ratio OH-itraconazole/ itraconazole increases considerably during multiple dose administrations and the difference between OH-itraconazole and itraconazole levels becomes more pronounced with increasing numbers of doses. Our study provides data on a single dose administration of itraconazole and the results may not be compared or extrapolated to multiple dose or steady-state pharmacokinetics.

The question remains as to whether monitoring of itraconazole and OH-itraconazole levels is really of clinical importance. A safe range for itraconazole drug levels has not been defined yet, and because of its relatively minimal side effects a narrow range is unlikely.[17] However, information on efficacy of itraconazole in the clinical setting is largely dependent upon clinical judgement. Moreover, variability in itraconazole levels within and between patients is high and itraconazole pharmacokinetics is unpredictable. Therefore, itraconazole drug level monitoring may be useful in selected patients, for example in those who receive concurrent CYP inducers or inhibitors.[17] In patients with a life-threatening fungal infection, or in those who are not responding to treatment, measuring of itraconazole concentrations may be used to verify the existence of therapeutic levels of the drug.[16]

In conclusion, our results show a highly variable, but unaltered exposure to itraconazole and an increased exposure to OH-itraconazole when itraconazole is administered in combination with CsA. Despite an increased understanding of the pharmacokinetic mechanisms underlying the interaction profile of itraconazole and CsA, it is still difficult to discern conclusively the importance and impact of the various metabolic processes that are involved in this interaction.

Acknowledgements

This study was supported by an unrestricted grant from Ortho Biotec, a division of Janssen-Cilag Pharmaceuticals, Tilburg, The Netherlands.

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Chapter 7

Cefpirome as empirical treatment for febrile neutropenia in patients with haematological malignancies.

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Published in a shortened version in Haematologica 2005;90:1005-1006.

Abstract

Cefpirome and other fourth generation cephalosporins are used with increasing frequency for the empirical treatment of febrile neutropenia. The purpose of this study was to assess the clinical efficacy of cefpirome and its activity against isolated pathogens in neutropenic patients with haematological malignancies. In addition, pharmacokinetics and optimal dosing regimen of cefpirome in this population were investigated.

In an open-label, clinical cohort study cefpirome 2 g twice daily was administered during 154 episodes of febrile neutropenia in 106 patients with haematological malignancies. Patients were evaluated for causes of fever and clinical outcome. Susceptibility patterns of isolated pathogens were determined. Cefpirome serum levels were measured in a subgroup of 24 patients.

Causes of fever were microbiologically documented infections (MDI) in 55 (36%) episodes, clinically documented infections (CDI) in 40 (26%) and fever of unknown origin (FUO) in 59 (38%) episodes. In 81 episodes (53%) the patient survived the neutropenic period without the need of treatment modification. Susceptibility testing of isolated pathogens showed adequate coverage of a broad range of Gram-positive and Gram-negative microorganisms, including viridans group streptococci, coagulase-negative staphylococci, *Enterobacteriaceae* and *Pseudomonas aeruginosa*. Our pharmacokinetic data indicate that a dosing regimen of cefpirome 2 g twice daily resulted in serum levels greater than 4 mg/l for 87.5% of the time and therefore may be sufficient in this population.

Cefpirome 2 g twice daily, proved to be a valuable addition to the therapeutic arsenal available for febrile neutropenia.

Introduction

The strategy of prompt intravenous administration of broad-spectrum antibacterial agents to patients who develop fever during profound neutropenia has become a generally accepted approach.[1-3] This empirical administration of antibiotics has played a crucial role in the reduction of morbidity and mortality due to infectious complications during neutropenia, as observed over the past decades. For years, the combination of a beta-lactam antibiotic and an aminoglycoside was the principal choice.[1] This combination offers possible synergism and a reduced risk of emergence of resistant strains. However, disadvantages of this regimen have become clear. These include a poor activity against some Gram-positive bacteria such as *Staphylococcus aureus*, coagulase-negative staphylococci and viridans group (VG) streptococci, which have become important pathogens in neutropenic patients. Moreover, there is a seriuos risk of aminoglycoside related adverse effects, such as nephrotoxicity and ototoxicity.

With the introduction of third and fourth generation cephalosporins with anti-Pseudomonas activity, the carbapenems and newer quinolones, single agent therapy has become a viable option.[1,3] Cefpirome is a fourth generation cephalosporin with greater beta-lactamase stability than the third generation cephalosporins.[4] The drug possesses enhanced activity in vitro against a broad range of Gram-positive microorganisms, including *Streptococcus pneumoniae*, VG streptococci and methicillin susceptible *Staphylococcus aureus*. Cefpirome is also highly active against *Enterobacteriaceae* and a variety of other Gram-negative species.[5] The drug is generally well tolerated and it is claimed that its pharmacokinetics are compatible with a twice daily dosing regimen.[5-7] In view of these properties cefpirome might be a suitable alternative for the empirical treatment of fever in neutropenic patients. Open trials, as well as some randomized studies have shown promising results.[8-10]

The dosage of cefpirome, recommended by the manufacturer for infections in neutropenic patients is 2 g twice daily. However, though pharmacokinetics have been investigated in healthy volunteers, data in patients treated for cancer are lacking.[5,11] Results obtained from pharmacokinetic evaluations in critically ill patients suggest that the administration of cefpirome twice daily produced low plasma trough levels in a number of patients and therefore may be inadequate.[12] As yet, the question regarding the optimal dosing regimen in patients treated for haematological malignancies remains to be answered.

We performed an open label, non-randomized clinical study to monitor the implementation of cefpirome for the empirical treatment of fever during neutropenia in patients with haematological malignancies. This study was designed to assess the clinical efficacy of cefpirome and its activity against isolated pathogens in neutropenic patients with haematological malignancies. In addition, pharmacokinetics of cefpirome in this population and the optimal dosing regimen of cefpirome in this population were investigated.

Patients and methods

Patients

Patients with haematological malignancies, admitted to the haematology ward of the VU University Medical Centre, Amsterdam, The Netherlands, in a two year period, were eligible if they became febrile while being neutropenic. Fever was defined as two consecutive axillar temperatures above 38.5°C, taken with an interval of at least one hour and unrelated to medication or blood products. Neutropenia was defined as an absolute neutrophil count (ANC) of less than 0.5 x 10^9/l. Duration of neutropenia was defined as the time between start of chemotherapy and neutrophil recovery (ANC >0.5 x 10^9/l). Patients were excluded if they had received antibacterial or antifungal agents within 72 hours before study entry. Systemic antimicrobial prophylaxis was allowed, which regularly comprised oral ciprofloxacin 500 mg two times daily, oral azithromycin 250 mg once daily and oral fluconazole 50 mg once daily, combined with nasal amphotericin B, 2 mg three times daily, nebulized into both nasal orifices. Adjustments of this prophylactic regimen were made if indicated by the results of weekly surveillance cultures of throat and anus.

Evaluations at baseline and cefpirome administration

When a patient fulfilled the entry criteria a complete history was taken and physical examination as well as a routine chest X ray, one urine culture and at least two blood cultures were performed. At least one blood culture was drawn from a peripheral vein and if appropriate a second culture sample was taken from a central venous catheter. Cultures from other suspected sites of infection were performed as clinically indicated.

After the initial evaluation patients received cefpirome (Cefrom®, Hoechst Marion Roussel, Hoevelaken, The Netherlands) 2 g two times daily, administered intravenously in 15-minute infusions. Follow-up studies included daily clinical evaluations, monitoring of body

temperature and repeated blood cultures in case of persisting fever. Chest or sinus radiographs were performed as clinically indicated. The administration of antibiotics was continued until the ANC was >0.5 x 10^9/l and complete resolution of all signs of infection was achieved.

Clinical and microbiological evaluations

Every febrile episode was classified in one of the following categories. A microbiologically documented infection (MDI) was diagnosed if appropriate cultures proved positive. A clinically documented infection (CDI) was established if cultures remained negative but appropriate signs or symptoms were present on clinical evaluation or radiography, which disappeared together with the fever. The remaining cases of fever were documented as of unknown origin (FUO). The evaluation of antimicrobial susceptibility to cefpirome and other antibiotics used, was performed according to standard microbiological techniques, using an in vitro disk diffusion test, which classified strains as sensitive (S), intermediate susceptible (I) or resistant (R).

Success of study treatment

Cefpirome treatment was considered to have been successful if the patient survived the episode of fever and neutropenia without any modification of the cefpirome regimen, and without signs of remaining active infection. Treatment was considered to have failed if a patient died during the study period, if one or more antibacterial or antifungal agents had to be added to the cefpirome regimen, or if cefpirome had to be replaced by other, more appropriate antimicrobial treatment. The latter either based on available microbiological culture data or because of unacceptable toxicity.

Pharmacokinetic evaluations

Assessment of pharmacokinetic parameters of cefpirome was performed in 24 subjects, randomly selected from the total of 154 patients entered in the study. Cefpirome serum levels were measured in a subgroup of 24 patients, randomly selected from the total of 106 patients entered in the study. All patients received cefpirome 2 g two times daily, until the final day of treatment. On the day that cefpirome normally would have been stopped the standard dosing regimen was switched to one of three different dosing regimens. Group I (n=8) continued to receive cefpirome 2 g twice daily, group II (n=8) received cefpirome 1 g three times daily and group III (n=8) received a single dose of 500 mg followed by 3 g cefpirome continuously i.v. From all patients venous blood was collected immediately prior to and after the first

administration of 2 g cefpirome at the start of cefpirome treatment, at t = -0.25, 0, 0.5, 1, 3, 6, 9 and 12 hours. Subsequently, venous samples were drawn at the final 24 hours of cefpirome treatment, during the three different dosing regimens, at t = -0.25, 0, 0.5, 1, 3, 6, 12, 18 and 24 hours.

Venous blood samples were centrifuged immediately after collection and the plasma was stored in plastic tubes at –30°C until analysis. Samples were analysed by reversed phase high-performance liquid chromatography (HPLC) with diode array detection (Gynkotek, Germering, Germany). The plasma cefpirome concentration versus time data were fitted to a two-compartment model using the WinNonlin software package (Scientific Consulting), yielding the pharmacokinetic parameters elimination half-life ($T_{½}$), area under the curve extrapolated to infinity (AUC_{inf}), total body clearance (Cl), and volume of distribution at steady state (Vss). The model was used to predict the duration of time between cefpirome administration and the moment that the serum concentration dropped below 4 mg/L, for each of the different dosing regimens. The concentration of 4 mg/l, being the MIC_{50} and a breakpoint of *Pseudomonas aeruginosa* was defined as a target concentration to be exceeded.[5,12]

Statistical analysis

Differences in time to defervescence between the MDI, CDI and FUO group were analysed using the Kaplan-Meier method. The results of pharmacokinetic parameters were compared by means of the log-rank test. The P-values are two sided.

Results

Study population

A total of 154 neutropenic episodes with fever were studied, which occurred in 106 eligible patients. Patient characteristics, including disease and treatment variables are given in Table 1. All patients had profound granulocytopenia at the onset of fever. Mean duration of neutropenia, calculated from the start of chemotherapy, was 24 days, with a range of 4 to 68 days. Patients received cefpirome for a mean of 9.8 days, with a range of 1 to 42 days. Fifty-two of 106 patients had acute leukaemia. In 58 of 154 (38%) episodes autologous peripheral blood stem-cell transplantation was part of the treatment.

Table 1. Characteristics of 154 neutropenic episodes in 106 patients.

Variable	Number *n* (%)
General characterisics	
Number of neutropenic episodes	154
Number of patients	106
Male/ female	63/ 43 (59/ 41)
Age (years, mean ± SD)	50.2 ± 13.6
Diagnoses (in 106 patients)	
Acute myeloid leukemia	44 (41)
Acute lymphoblastic leukemia	8 (7)
Myelodysplastic syndrome	6 (6)
Lymphoma	21 (20)
Multiple myeloma	20 (19)
Chronic myeloid leukemia	6 (6)
Hairy cell Leukemia	1 (1)
Disease activity (in 154 neutropenic episodes)	
Active disease	71 (46)
Partial remission	38 (25)
Complete remission	43 (28)
Not evaluable	2 (1)
Treatment (in 154 neutropenic episodes)	
Autologous stem cell transplantation	58 (38)
Central venous access catheter	148 (96)
Neutropenic episode[a] (days, mean ± SD)	24.8 ± 9.5
Duration of fever (days, mean ± SD)	4.5 ± 4
Duration of fever (days, range)	1-25
Cefpirome	
Treatment days (mean ± SD)	9.8 ± 6.2
Treatment days (range)	1-42

a. Duration of the neutropenia is calculated from the start of chemotherapy, absolute neutrophil count (ANC) <0.5 x 10^9/l.

Causes of fever

Patients were evaluated for causes of fever. A microbiologically documented infection (MDI) was evidenced in 55 (36%) of episodes, a clinically documented infection (CDI) in 40 (26%) and fever of unknown origin (FUO) in 59 (38%) episodes (Table 2). In the majority of cases an MDI was evidenced by a positive bloodculture(40/55), while in cases with CDI signs and symptoms of pneumonia (14/40) and sinusitis (10/40) were most frequently found.

Table 2. Causes of fever in 154 neutropenic episodes.

Causes of fever	Number *n* (%)
Microbiologically documented infections[a]	55 (36)
Blood	40
Sputum	10
Urine	3
Sinus	1
Skin, ulcus	3
Perineum	1
Clinically documented infections	40 (26)
Skin, insertion site catheter	3
Lungs	14
Oral cavity	7
Otitis	1
Sinus	10
Oesophagus	1
Perineum	2
Genito-urinary tract	2
Fever of unknown origin (FUO)	59 (38)

a. In some patients more than one microorganism was isolated from different sites at the same time.

Microbiological evaluation

Culture data of the MDI episodes are given in Table 3. Blood cultures predominantly revealed Gram-positive microorganisms, including VG streptococci and coagulase-negative staphylococci. In sputum cultures Gram-negative bacteria predominated. Susceptibility patterns showed that most Gram-positive cocci were susceptible to cefpirome. However, all isolates of enterococci (*E. faecium* and *E. faecalis*), one strain of *S. sanguis* and one strain of *S. adjacens* were found to be resistant to cefpirome. In addition, *Corynebacterium jeikeijum* was not susceptible. Considering Gram-negative microorganisms, all strains of *E. coli* and *Serratia marcescens* and most strains of *Pseudomonas aeruginosa* were susceptible to cefpirome *in vitro*. Intermediate susceptibility or resistance to cefpirome was found in all cases of *Stenotrophomonas maltophilia* and in one case of *Pseudomonas aeruginosa*.

Clinical outcome and response to the study drug

Among all febrile episodes treatment was successful in 81 (53%) of 154 episodes. The rate of success was largest in the FUO episodes, 45/59 (76%), 21/40 (53%) in the CDI episodes, and smallest in the MDI episodes 15/55 (27%). The time to resolution of fever in the FUO episodes was significantly shorter than in the MDI and CDI episodes. Median duration of

fever in the FUO episodes was 3 days and in the MDI and CDI episodes both 4 days (P = 0.001, by the log-rank test).

Table 3. Isolated microorganisms and susceptibility to cefpirome in 55 episodes with a microbiologically documented infection (MDI).

Causes of fever	No. of episodes	No. of strains Sensitive (S), Intermediate (I) or Resistant (R) to cefpirome.		
		S	I	R
Microbiologically documented infections	**55 (36%)**			
Bloodstream infections	40			
Coagulase negative staphylococci	10	10		
Viridans group (VG) streptococci	16	14		2
Enterococci	7			7
Corynebebacterium jeikeijum	3			3
Pseudomonas aeruginosa	2	2		
Candida tropicalis	1			
Other	1			1
Respiratory tract infections	10			
Pseudomonas aeruginosa	3	2	1	
Stenotrophomonas maltophilia	2		1	1
Serratia marcescens	2	2		
E. coli	1	1		
Aspergillus fumigatus	2			
Urinary tract infections	3			
Enterococci	2			2
Proteus mirabilis	1	1		
Other	2	1		1
Clinically documented infections	**40 (26%)**			
Lungs	14			
Ear, nose, throat	8			
Sinuses	10			
Skin	3			
Genital or (peri) anal	4			
Other	1			
Fever of unknown origin (FUO)	**59 (38%)**			

The majority of failures, both in the MDI and CDI episodes were classified as such because of the addition of another antimicrobial agent. Vancomycin was added to cefpirome during 23 episodes. This frequently occurred if blood cultures revealed Gram-positive cocci and the treating physician was concerned about possible infections with resistant enterococci or coagulase negative staphylococci. However, subsequent identification of the microorganisms

involved frequently showed bacteria with adequate susceptibility to cefpirome, including VG streptococci and coagulase negative staphylococci. In addition, vancomycin was added if culture-data revealed the presence of *Corynebacterium* spp, or *Enterococcus* spp. Amphotericin B was added during 17 febrile episodes. This was because of persisting fever after 4x24 hours (11/17) or if signs of a possible fungal infection occurred (6/17). One *Candida tropicalis* infection was microbiologically documented; other episodes were probable or possible fungal infections. In 14 febrile episodes cefpirome was replaced by vancomycin/ aztreonam because of a moderate to severe skin rash, probably caused by the use of cefpirome. No other toxicities of the study treatment were observed that necessitated change of the cefpirome treatment regimen. Imipenem-cilastatin in combination with tobramycin was initiated in 5 episodes, since cultures revealed the presence of *Pseudomonas aeruginosa*.

Table 4. Outcome rates of cefpirome treatment.

Outcome	No. of episodes	Outcome according to causes of fever		
		CDI (n=40)	MDI (n=55)	FUO (n=59)
Success (%)	81 (53)	21 (53)	15 (27)	45 (76)
Failure (%)	73 (47)	19 (47)	40 (73)	14 (24)
Death				
Death due to uncontrolled infection	4	1	3	
Death due to non-infectious causes	3	1	1	1
Recurrent fever within 4 days	3		3	
Modification of treatment required				
Addition of Vancomycin	23	2	20	1
Addition of Amphotericin B	17	7	6	4
Addition of Metronidazole	2	1	1	
Addition of other antimicrobial agent	1		1	
Switch to Vancomycin/ Aztreonam	14	5	1	8
Switch to Imipenem/Tobramycin	5	2	3	
Switch to other antimicrobial agent	1		1	

Mortality

Seven patients died during the study period, four of whom still had fever at the time of death (MDI n=3, CDI n=1). One had nodular pulmonary infiltrates and sputum cultures proved to be positive with *Aspergillus fumigatus*. Three other patients with fever had massive pulmonary infiltrates; responsible microorganisms could not be identified, in one patient

pulmonary embolism was found on post-mortal examination. Three patients died while afebrile, one due to massive cerebral hemorrhage, 2 patients died of disease progression.

Pharmacokinetic evaluation

Pharmacokinetic data were obtained from 24 patients (Group I, II, III). A total of 476 venous blood samples were drawn. Two patients were not evaluable. One patient in the group receiving 2g cefpirome twice daily (group I) was excluded from analysis because of dose-protocol violations during the 2nd sample period. Another patient in the group of patients receiving 1g cefpirome three times daily (group II) died during the sample period. Because the protocol dictated the collection of venous blood samples prior to and after the first dose of 2 g cefpirome in all patients (group I, II and III), a total of 22 sample periods were evaluable for the 2 g dose. Data are given in Table 5. The regimen of 2 g cefpirome administered twice daily (Group I) resulted in serum concentrations greater than the pre-defined target concentration of 4 mg/l during a mean of 87.5% of the time (*t*). This was not significantly different from the patients that received cefpirome three times daily during the last 24 hours of treatment (Group II), who had serum concentrations >4 mg/l for 92.6% of the time (P = 0.4). The patients receiving a 500 mg loading dose followed by continuous i.v. administration of 3 g cefpirome (Group III) had serum concentrations >4mg/l throughout the entire sampling period, which is significantly better than the 2 g twice daily regimen (P = 0.01).

Table 5. Pharmacokinetics of cefpirome in a subset of 22 patients, receiving three different dosing regimens of cefpirome.

Reference	No. of patients (n)	Dose (g) interval (h)	$T_{1/2}$ (h)	AUC (mg.h/l)	Cl (ml/min)	V_{ss} (l)	*t* >4.0 mg/l (%)
Present study							
Group I,II,III	22	2g q 12 h	3.2	377	92	18.4	87.5
Group II	7	1g q 8 h	3.0	240	87	16.3	92.6
Group III	8	0,5 g loading dose, 3 g c.i.	3.3	132	70	14.6	100
Lipman et al. (12) Critically ill	12	2g q 12 h	2.5	266	130	24	67
Nakayama et al. (11) Healthy volunteers	6	2 g q 12 h	1.7	259	133	15.7	

$T_{1/2}$ = elimination half-life; AUC = area under the plasma concentration-time curve; Cl = total body clearance; V_{ss} = apparent volume of distribution at steady-state; *t* >4.0 mg/l = % of time that serum concentration of cefpirome is above 4 mg/l; ci = continuous infusion.

Discussion

Cefpirome is a fourth generation cephalosporin, with a broad spectrum of antibacterial activity, better activity against Gram-positive organisms and greater stability to beta-lactamases than the third-generation cephalosporins. Moreover, a twice daily dosing regimen may be sufficient.[5] Therefore, cefpirome monotherapy may serve as a suitable alternative in treatment regimens for infections in neutropenic patients.[1] In comparative trials cefpirome has shown equivalent efficacy and safety to ceftazidime and piperacillin-tazobactam, in the treatment of suspected bacteraemia or sepsis and febrile neutropenia.[8,9]

In this open label, non-randomized clinical trial we administered cefpirome 2g twice daily during 154 episodes of febrile neutropenia, occurring in 106 patients. We were able to identify the cause of fever in 62% of febrile episodes. In general FUO accounts for approximately 50% of causes of fever in neutropenic patients.[2] Strict adherence to the investigational protocol may have contributed to the relatively low percentage of FUO (38%) as observed in our study.

In 81 of 154 episodes the patient survived the episode of neutropenia and fever without any modification of the cefpirome regimen (overall rate of success 53%). This is slightly lower than the reported success rates of cefpirome in comparable clinical settings and also lower than the rate of success achieved with imipenem-cilastatin, as previously reported by our group and others.[8,9,13,14] This finding may be explained by the fact that our protocol provided rather liberal guidelines for the addition of other antibiotics to cefpirome. Physicians were reluctant to rely on cefpirome monotherapy and frequently prescribed vancomycin as soon as baseline culture data revealed Gram-positive microorganisms, in view of possible infection with enterococci or coagulase negative staphylococci. However, in a number of cases further determination of these bacteria showed adequate susceptibility to cefpirome. Vancomycin was discontinued subsequently, but as defined by the protocol these patients were categorized as failure.

Treatment with cefpirome proved to be most successful in FUO episodes, in which resolution of fever without treatment modification was achieved in 76%. CDI episodes were successfully treated in 53% and the lowest rate of success was observed in the MDI episodes (27%). This is in line with results from large-scale clinical studies on antimicrobial treatment in neutropenic patients.[2,15,16] Patients with an obvious focus of infection clearly represent a population that is more difficult to treat than those without any focus at all. Infectious deaths occur in one-fifth of episodes with a focus of infection, in comparison with less than 5% for

episodes without one.[2,15] In our study 4 patients died due to uncontrolled infection, 3 with MDI and one with CDI. No deaths occurred among patients with FUO.

As observed previously, Gram-positive bacteria predominated the isolated microorganisms (72%).[8,14,17] Most strains of VG streptococci and coagulase-negative staphylococci were susceptible to cefpirome. In vitro studies have shown that up to 100% of strains of beta-hemolytic streptococci may be expected to be susceptible, and 80-90% of coagulase-negative staphylococci.[10,18] In our study, enterococci were resistant to cefpirome. These findings are in contrast with data from literature. About 70% of strains of *E. faecalis* are reported to be susceptible to cefpirome in vitro. MIC values for enterococci however, are certainly not low and vary between 4 and 8 mg/l.[5,10,18] This observation underscores the importance of gaining knowledge of local resistance patterns of specific pathogens, especially if borderline susceptibility is expected. *Corynebacterium jeikeijum* was resistant to cefpirome. These Gram-positive rods frequently display multi-resistance, though in general they remain susceptible to vancomycin.

Considering Gram-negative bacteria, cefpirome was active against E. coli and other *Enterobacteriaceae*, including *Serratia* and *Proteus* species. MIC values for these microorganisms are reported to be very low, and vary between 0.4 and 2 mg/l. [5,10,18] *Pseudomonas aeruginosa* was susceptible to cefpirome, except for one strain with intermediate susceptibility. In an epidemiological survey in Intensive Care and Haematological units from 12 large hospitals in the Netherlands, including ours, 82% of isolated Pseudomonas strains were found to be susceptible to cefpirome.[10] However, in other studies MIC_{90} values between 12.5 and ≥32 mg/l have been reported, indicating borderline susceptibility or resistance.[5,18] Despite our findings, it is probably right to state that cefpirome has only moderate activity against *Pseudomonas aeruginosa*. In our study, physicians changed cefpirome to imipenem-cilastatin in combination with tobramycin, according to local protocols, if *Pseudomonas aeruginosa* was suspected or isolated. This strategy may be debated, considering the majority of *Pseudomonas* strains being susceptible to cefpirome, addition of an aminoglycoside might have been a viable option.

We are the first to report pharmacokinetic parameters of cefpirome in patients with haematological malignancies. Cefpirome pharmacokinetics have been studied extensively in healthy volunteers and in patients with renal impairment. The plasma half-life of cefpirome and the time that the serum levels exceed the MIC of the common pathogens are sustained enough to recommend a twice-daily dosing regimen.[5-7,19] However, in a study in critically ill patients low trough levels were found, following intravenous administration of 2 g cefpirome

twice daily.[12] The authors suggested that this drug regimen may be inadequate in critically ill patients. Most likely the difference in pharmacokinetics as compared with healthy volunteers is explained by an increase of volume of distribution, due to extensive fluid therapy in critically ill patients. This may also hold true for neutropenic patients, who are treated with intensive chemotherapy and receive frequent intravenous drug and fluid administrations. Our data however, show that a twice daily dosing regimen of 2 g cefpirome resulted in serum levels greater than 4 mg/l for 87.5% of the time. This may be explained by our finding that the volume of distribution of cefpirome in neutropenic subjects is comparable with those found in healthy volunteers, rather than in critically ill patients.[12] Cefpirome 2 g twice daily is recommended by the manufacturer as a dosing regimen for infections in neutropenic patients and our data confirm that this may be adequate. However, alternative dosing schedules of 1g three times daily or a loading dose of 500 mg followed by 3g continuously i.v may even be better, with serum concentrations above 4 mg/l for 92.6 and 100% of the time respectively.

In common with most other beta-lactam antibiotics cefpirome was well tolerated. In 14 (9%) episodes cefpirome was changed to vancomycin/ aztreonam because of skin rash, possibly due to cefpirome. Most likely concomitantly prescribed medications are at least in part responsible for this relatively high number of failures. In literature, the most common adverse event is diarrhoea, reported in 1.6% of patients. Rash occurred in 1.4%.[4,20] Adverse events are more frequently reported in patients who were seriously ill, and particularly those with neutropenia.[20]

In conclusion, cefpirome proved to be a valuable addition to the therapeutic arsenal available for febrile neutropenia to date. The drug has broad Gram-positive and Gram-negative coverage, but gaps in the antimicrobial spectrum involve enterococci, Corynebacterium spp. and *Stenotrophomonas maltophilia*. Moreover, a limited activity against *Pseudomonas aeruginosa* may in the long run hamper its use as a single agent therapy. Our pharmacokinetic data indicate that in neutropenic subjects a dosing regimen of cefpirome 2 g twice daily intravenously is sufficient. Finally, it should be emphasized that extensive use of single-drug therapy requires vigilance, and close monitoring of the local microbial flora, since success is largely dependent upon a continued susceptibility to the drug involved.

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Chapter 8

Outbreak of vancomycin-resistant *Enterococcus faecium* in a haematology unit: risk factor assessment and successful control of the epidemic.

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British Journal of Haematology 2002;116:826-833.

Abstract

We describe an outbreak of vancomycin-resistant *Enterococcus faecium* (VRE) on the haematology ward of a Dutch university hospital. After the occurrence of three consecutive cases of bacteraemia with VRE, strains were genotyped and found to be identical. During the next 4 months an intensive surveillance programme identified 21 additional patients to be colonized with VRE, while two more patients developed bacteraemia. A case-control study was carried out to identify risk factors for VRE acquisition. In comparison with VRE-negative control patients (n = 49), cases (n = 24) had a longer stay on the ward during the year preceding the outbreak (25.8 versus 10.1 days, P = 0.02), more cases with acute myeloid leukaemia (11 versus 4, odds ratio (OR) 9.5, 95% confidence interval (CI_{95}) 2.4-32.2) and higher grades of mucositis (P = 0.03). Logistic regression analysis identified antibiotic use within 1 month before admission (OR 13.0, CI_{95} 2.1-80.5, P = 0.006) and low albumin levels at baseline (OR 1.2, CI_{95} 1.1-1.3, P = 0.02) to be independent risk factors. Four patients with VRE-bacteraemia were successfully treated with quinupristin/dalfopristin (Synercid®). Control of the outbreak was achieved by step-wise implementation of intensive infection control measures, which included the cohorting of patients, allocation of nurses and reinforcement of hand hygiene.

Introduction

With the emergence of resistance to glycopeptides, enterococci have rapidly become important nosocomial pathogens.[1] Over the past decade, vancomycin-resistant enterococci (VRE) have been reported with increasing frequency as the cause of nosocomial outbreaks, usually involving high-risk patient populations, such as haemato-oncology patients, organ transplant recipients and patients in intensive care units.[2-8] In general, such outbreaks have a serious impact on the daily care and treatment of patients on the ward or in the hospital involved. Extensive infection control measures are mandatory to prevent further spread of the microorganism and sometimes the ward even has to be temporarily closed to new admissions. More important, VRE may cause life-threatening systemic infections in immunocompromised patients. Until recently, effective antibiotic strategies were lacking and systemic infections with VRE in these patients have been associated with in-hospital mortality rates ranging from 40-100%.[9,10] Established risk factors for VRE colonization or infection include neutropenia, prolonged hospitalization, the frequent use of multiple broad-spectrum antibiotics and the use of invasive procedures.[2,3,5] Therefore, it is not surprising that patients with haematological malignancies are at a predominant risk for nosocomial acquisition of VRE. Many reports have included haemato-oncology patients in their analysis of VRE acquisition. However, only a few have studied strictly haematological populations.[11-13]

In 1999 a VRE outbreak occurred on our haematology ward. A case-control study was carried out to identify possible risk factors associated with VRE acquisition. Molecular biological techniques were used to genotype the VRE isolates. Moreover, successful control of the outbreak was achieved after step-wise implementation of intensive infection control measures, which we describe in detail.

Materials and methods

Setting

The outbreak occurred on the 21-bed haematology ward of the VU University Medical Centre, Amsterdam, the Netherlands. The haematology department is the referral centre for adult patients with haematological malignancies from the north-western part of the Netherlands. The unit comprises two private rooms and four rooms with a four-bed capacity. Patients share a limited number of toilet facilities, two for women and two for men, located in

one sanitary unit. In addition the ward is equipped with three private rooms with controlled-airflow facilities. According to local protocols, all patients on the ward, who were expected to be neutropenic (absolute neutrophil count $<0.5 \times 10^9$/l) for more than 10 d, received ciprofloxacin 500 mg b.i.d., azitromycin 250 mg b.i.d. and fluconazole 50 mg daily as infection prophylaxis. Standard care of febrile neutropenic patients on the ward included empirical treatment with cefpirome 2 g b.i.d. (Cefrom®), a broad-spectrum cephalosporin. Vancomycin 1 g b.i.d. was added to the antibiotic regimen if culture data so dictated. If no defervescence occurred within 96 h, amphotericin B 0.7 mg/kg was given intravenously.

Microbiological surveillance

Between November 1998 and May 1999, three patients admitted to the haematology ward developed bloodstream infections with a VRE strain, which proved to be clonally related. This prompted microbiological screening of all patients in the ward by biweekly culture of anal swab specimens. Additionally, rectal swabs were obtained from all newly admitted patients upon admission and twice weekly thereafter. Environmental samples were taken from patient-related equipment, housekeeping articles and surfaces of furniture and other room equipment.

Microbiological methods

Anal swab specimens and environmental swab specimens were cultured for the detection of VRE in Enterococcosel broth (BBLTM, Becton & Dickinson, Sparks, USA). After incubation overnight at 37°C, black-coloured broth media were subcultured on Enterococcosel agar (BBLTM, Becton & Dickinson) for 48 h at 37°C. Black colonies were identified at the species level using standard microbiological methods. Susceptibility tests were performed using the disk diffusion method (Rosco tabs, A/S Rosco, Taastrup, Denmark) on Mueller Hinton agar (Difco, Becton & Dickinson). Vancomycin and teicoplanin resistance was confirmed with Minimal Inhibitory Concentration (MIC) determination using the E-test (AB Biodisk, Solna, Sweden). Resistance was defined as MIC $\geq$32 mg/l.

Molecular typing

All isolates were fingerprinted by amplified fragment length polymorphism (AFLP), which has been described in detail previously.[14] This technique was carried out using fluorescent primers.[15] Levels of similarity of fingerprints were analysed by Pearson correlation, with specialized software (Gel Compar 4.0, Applied Maths, Kortrijk, Belgium). A dendrogram of

percentage similarity was produced by the unweighted pair group method. Isolates with a homology of >90% were considered to be clonally related.

Case-control study

To identify risk factors for nosocomial acquisition of VRE, we performed a case-control study. Cases were defined as patients who became colonized or infected with the epidemic VRE strain. Controls were all patients who were hospitalized on the haematology ward during the outbreak period and did not acquire VRE. To be eligible, controls had to have at least one negative culture of a rectal swab specimen. If multiple admissions of one subject occurred during the outbreak period, the patient was included in the study only once to avoid disproportional contribution of particular cases or controls to the final analysis. To identify risk factors for colonization or infection with VRE, a standardized set of data was extracted from the medical records of cases and controls. Data collected included patient demographics, disease characteristics and treatment variables, antibiotic use and variables defining the clinical course. Data for cases were collected up to the day of the first culture that yielded VRE, and for controls for the total duration of their hospital stay.

Statistical analysis

Univariate analysis of categorical variables was performed by calculating odds ratios (OR) and 95% confidence intervals (CI_{95}). In case of categorical variables with an ordering or grading scale, the chi-square test for trend was used. Student's t-test was used for comparison of means. Logistic regression analysis was performed to identify independent risk factors for VRE acquisition. All tests were two-tailed, alpha was set at 0.05.

Results

Description of the outbreak

Between November 1998 and May 1999 three patients admitted to the haematology ward developed VRE bacteraemia. Molecular typing of these VRE showed that the strains were clonally related. The subsequent microbiological surveillance programme showed that, between June and September 1999, 21 additional patients acquired the epidemic VRE strain, resulting in a total number of VRE-positive patients of 24. In addition to the first three patients with VRE bacteraemia, two patients who became colonized with VRE subsequently

developed VRE bloodstream infection. The outbreak was reported to the Health Care Inspectorate of the Ministry of Health, Welfare and Sport. The epidemic curve is shown in Figure 1.

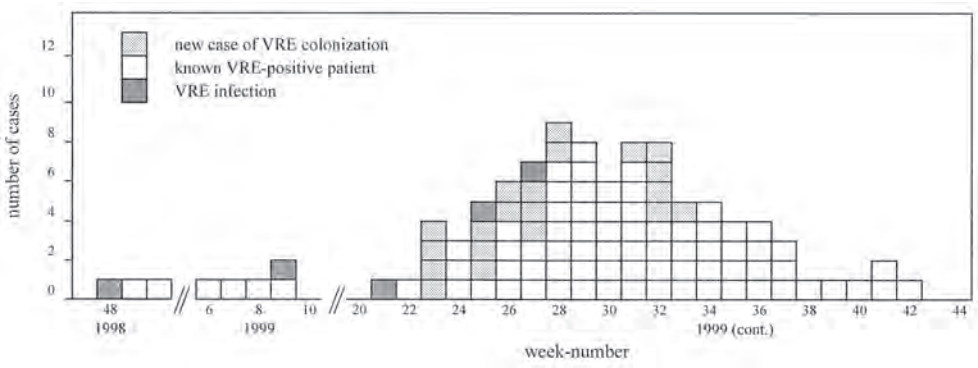

Figure 1: Epidemic curve, showing the weekly incidence and prevalence of VRE cases. Each patient present on the ward, known to be VRE-positive, is represented by one square.

Infection control procedures

The first attempt to control the outbreak consisted of isolation precautions for all identified VRE carriers. Gowns and gloves had to be worn upon entering patient rooms and during every examination or other procedure involving contact with the patient. Weekly meetings between medical, nursing, microbiological and infection-control staff were instituted. All medical and paramedical personnel were educated and hand hygiene was reinforced. The use of the ward as a thoroughfare to the adjacent oncology ward was discouraged by placing warning signs at the entrances. Floors and horizontal surfaces were disinfected daily.

With the ongoing identification of new cases, infection control measures were extended. Cefpirome, a broad-spectrum cephalosporin used as a standard for the empirical treatment of neutropenic fever, was replaced by imipenem-cilastatin and the use of vancomycin was restricted. The three portable blood-pressure devices used on the ward were replaced by sphygmomanometers fixed to the wall, one for every patient. Moreover, a private portable toilet facility was allocated to each individual patient. In week 31 a patient who had been admitted to the ward before the outbreak period was re-admitted. He appeared to be VRE-negative on initial screening, but was found to be colonized with the epidemic VRE shortly thereafter. Five VRE-negative patients who had shared the room with this patient became colonized with the epidemic strain as well. This finding prompted the subdivision of all patients into three predefined cohorts. The first cohort consisted of all 'known VRE-positive'

patients. Additionally, patients who had been hospitalized between November 1998 and August 1999 were considered 'possibly VRE-positive', irrespective of culture results. They were cohorted and barrier nursed. No sharing of any article between patients was allowed in this patient group. New patients, who had never been admitted to the haematology ward before, were considered to be a 'true VRE-negative' cohort, and were nursed in separate rooms, without isolation precautions. On every shift nurses were strictly allocated to either VRE-positive or -negative patients. Clinicians conducted their rounds visiting the VRE-negative patients first, followed by the 'possibly colonized patients' and the VRE-positive patients last. In the third week of August, week 33, the last patient colonized with the epidemic VRE was identified. Several 'known VRE-positive' patients were re-admitted thereafter. Occasionally, these patients had negative screening cultures on admission; however, subsequent VRE cultures invariably became positive again. The last patient known to be VRE positive was admitted in week 47. After his discharge at the end of December 1999, no known VRE-colonized patients were admitted and no new cases were identified. After resolve of the outbreak we continued with weekly VRE surveillance cultures as a standard infection control measure on our ward. Culture data have shown successful control of the epidemic to date.

Microbiological surveillance and environmental cultures

Between June 1999 and November 1999, 287 anal surveillance cultures were performed during 115 admissions on the haematology ward. Of these 287 cultures, 76 (26%) yielded vancomycin-resistant *Enterococcus faecium*, in 24 patients. All isolates manifested high-level resistance to vancomycin and teicoplanin (E-test MIC $\geq$32 mg/l), which is compatible with the VanA glycopeptide resistance phenotype. In addition the strains were resistant to amoxycillin, clindamycin and erythromycin. None of the culture samples obtained from 27 environmental sites were positive for VRE. However, at the time of this sampling, intensive infection control measures had already been installed. AFLP analysis was performed on all isolated strains, and showed that isolates had > 90% homology of DNA patterns (Figure 2). This finding was indicative of similarity of strains and provided ultimate proof of the nosocomial transmission of a single clone. Two patients had VRE isolates that proved to be unrelated to the epidemic strain (Figure 2, B1).

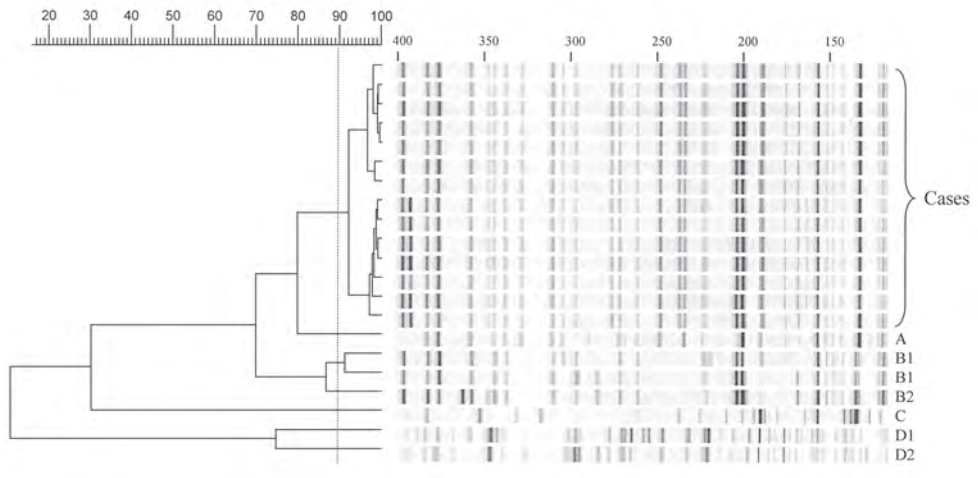

Figure 2: Digitized AFLP patterns and dendrogram of the first 14 vancomycin-resistant *Enterococcus faecium* outbreak isolates and of nonoutbreak isolates, *E. faecium* reference strain CDC ny2 (A), non-epidemic *E. faecium* isolates from 2 patients on the haematology ward (B1) and from a patient on another ward in the hospital (B2), *E. faecalis* CDC ny3 (C), *E. gallinarum* from a nearby local hospital (D1), and *E. gallinarum* CDC ny4 (D2). All strains were resistant to vancomycin. The scale represents percentage of homology, the cut-off value of identical strains is determined at 90% (dotted line). Molecular weights are shown above the lanes.

Case-control study

During the outbreak period a total number of 115 admissions of 84 patients was observed. Twenty-four cases were identified. Forty-two clinical episodes were excluded from the evaluation because of a second or third admission of the same patient (n = 31), because no VRE surveillance cultures were performed (n = 9) or because a non-epidemic strain of VRE was identified using AFLP techniques (n = 2). Forty-nine patients remained evaluable as controls.

Characteristics of the 24 case patients were compared with the 49 control patients by univariate analysis (Table 1). Cases and controls were similar in age and sex. There were no major differences between cases and controls in disease activity, as measured by the status of remission or in several treatment characteristics, including the use of stem-cell transplantation and the use of haematopoietic growth factors. Cases had stayed significantly more days on the ward during the year preceding the outbreak period and had received antibiotics more frequently than controls in the month prior to hospitalization. Significantly more case patients had acute myeloid leukaemia (AML) than control patients. Mean albumin levels at baseline and mean minimal albumin levels appeared to be lower in cases than in controls. Case

patients were suffering from higher grades of mucositis than control patients. Fever occurred more frequently in the case group and case patients were having more microbiologically documented infections, with a predominance of bloodstream infections. During the period before VRE acquisition, cases received both preventive and therapeutic antibiotics more frequently, including cephalosporins, vancomycin, imipenem-cilastatin and amphotericin B. Overall mortality did not differ significantly between the groups.

Variables that were significantly associated with VRE acquisition by univariate analysis and that were considered to be of clinical relevance were entered in a multivariate model (Table 2). Antibiotic use within 1 month before admission (OR 13.0 (CI_{95} 2.1-80.5), P = 0.006) and the mean albumin level at baseline (OR 1.2, CI_{95} 1.1-1.3, P = 0.02) appeared to be independently associated with VRE colonization or infection.

Cases with VRE bacteraemia

Bloodstream infections with VRE developed in five cases. Four patients were diagnosed with AML and one patient had refractory anaemia with excess blasts in transformation (RAEB-t). All patients had active disease on admission, except for one patient who was in complete remission.

The first patient with VRE bacteraemia received teicoplanin and rifampicin and recovered quickly after regaining a normal neutrophil count. In another patient blood cultures revealed VRE only shortly after his death. The remaining three patients and one additional patient with a non-epidemic VRE received quinapristin/dalfopristin (Synercid®) 7.5 mg/kg t.i.d. intravenously, which had not been officially registered in the Netherlands at that time. All four patients treated with quinapristin/dalfopristin had a favourable clinical and bacteriological response. Later, one patient died of respiratory failure of unknown origin, not related to VRE.

Discussion

Patients with haematological malignancies appear to be at a predominant risk for the acquisition of VRE, and nosocomial outbreaks involving these patients have been reported previously.[4,5,12,13] The presence of a haematological malignancy has even been identified as an independent risk factor for infection with VRE.[16]

Table 1. Univariate analysis of case-control data

Variable	Cases (n= 24)	Controls (n= 49)	OR (CI_{95})
Baseline characteristics			
Male	12 (50)	30 (61)	NS
Female	12 (50)	19 (39)	NS
Age (years ± SD)	43.8 ± 14.8	45.7 ± 15.0	NS
Days on ward before outbreak (days ± SD)	25.8 ± 28.1	10.1 ± 21.0	$P = 0.02$
Diagnoses			
AML	11 (46)	4 (8)	9.5 (2.6-34.9)
Myeloma	3 (13)	18 (37)	NS
ALL	2 (8)	4 (8)	NS
Lymphoma	2 (8)	17 (35)	0.2 (0.04-0.8)
CML	2 (8)	2 (4)	NS
CLL	1 (4)	1 (2)	NS
Hairy cell leukaemia	0 (0)	3 (6)	NS
MDS	3 (13)	0 (0)	NC
Disease activity			
Complete remission	6 (25)	8 (16)	
Partial remission	2 (8)	19 (39)	$P = 0.5$[a]
Active disease	16 (67)	22 (45)	
Treatment			
Flow chamber	4 (17)	8 (16)	NS
Stemcel transplant	6 (25)	18 (37)	NS
Autologous	4 (17)	14 (29)	NS
Allogeneic	2 (8)	4 (8)	NS
GCSF	4 (17)	8 (16)	NS
Steroid use	8 (33)	20 (41)	NS
Antibiotics before admission			
Within 1 month b.a.	15 (63)	6 (12)	11.9 (3.6-39.2)
Vancomycin within 1 month b.a.	3 (13)	1 (2)	NS
Clinical course			
Hospital stay (days ± SD)	25.6 ± 8.9	17.9 ± 10.5	$P = 0.003$
Stay before VRE acquisition (days ± SD)	13.3 ± 6.9	17.9 ± 10.5	$P = 0.03$
Diarhoea	16 (67)	10 (20)	7.8 (2.6-23.4)
Faecal incontinence	9 (38)	5 (10)	5.3 (1.5-18.3)
Urine incontinence	3 (13)	1 (2)	NS
Decubitus	3 (13)	3 (6)	NS
Non compliance with the preventive	5 (21)	7 (14)	NS
Nasogastric feeding	0 (0)	0 (0)	NS
Antacid medication	12 (50)	26 (53)	NS
Neutropenia (ANC $<0.5 \times 10^9$/L, days ± SD)	16.3 ± 11.5	9.9 ± 11.3	P = 0.03
Neutropenia before VRE acq. (days ± SD)	10.8 ± 9.9	9.9 ± 11.3	NS
Death	5 (21)	3 (6)	NS
Mucositis			
None	6 (25)	28 (57)	
Grade I	12 (50)	13 (27)	$P = 0.03$[a]
Grade II	3 (13)	6 (12)	
Grade III	3 (13)	2 (4)	

Table 1. *Continued*

Variable	Cases (n= 24)	Controls (n= 49)	OR (CI_{95})
Clinical chemistry			
Mean albumin level at baseline (g/L ± SD)	32.9 ± 5.9	37.8 ± 6.5	*P* = 0.003
Mean minimal albumin level (g/L ± SD)	25.3 ± 5.8	30.8 ± 6.6	*P* = 0.001
Infection parameters			
Fever (t > 38.5 ºC)	18 (75)	18 (37)	5.2 (1.7-15.4)
FUO	6 (25)	10 (20)	NS
Microbiologically documented infection	8 (33)	4 (8)	5.6 (1.5-21.3)
Clinically documented infection	4 (17)	5 (10)	NS
Colonization on surveillance cultures	18 (75)	24 (49)	3.1 (1.1-9.2)
Positive cultures of normally sterile sites	10 (42)	5 (10)	6.3 (1.8-21.5)
Infection site			
Blood	7 (29)	3 (6)	6.3 (1.5-27.3)
Lung	4 (17)	3 (6)	NS
Diverse	1 (4)	3 (6)	NS
Antibiotics			
Patients using antibiotics before VRE acq.	16 (67)	15 (31)	4.5 (1.6-12.9)
Number of antibiotics used (mean ± SD)	2.6 ± 1.2	1.3 ± 0.6	*P* = 0.001
Systemic antimicrobial prevention	21 (88)	31 (63)	4.1 (1.06-15.6)
Cefpirome	7 (29)	4 (8)	4.6 (1.2-17.9)
Vancomycin	5 (21)	2 (4)	6.2 (1.1-34.7)
Imipenem-cilastatin	10 (42)	8 (16)	3.7 (1.2-11.1)
Amfotericin-B	8 (33)	1 (2)	24.0 (2.8-207.0)
Tobramycin	2 (8)	1 (2)	NS
Metronidazole	2 (8)	0 (0)	NC

a. Chi-square test for trend.

Unless otherwise indicated, all values represent the number (%) of patients. Data representing variables 'before VRE acquisition' in cases are compared with data from the 'whole length of stay' for controls.

SD, standard deviation; b.a., before admission; NS, not significant; NC, not calculated; ALL, acute lymphoblastic leukaemia; CML, chronic myeloid leukaemia; CLL, chronic lymphocytic leukaemia; MDS, myelodysplastic syndrome; GCSF, granulocyte colony-stimulating factor; ANC, absolute neutrophil count; FUO, fever of unknown origin; VRE, vancomycin resistant *Enterococcus faecium*.

Univariate analysis of our case-control data showed a striking over-representation of patients with AML in the case group, which was also observed in other case series.[4,13] In general, patients with AML are known to be at a very high risk for infectious complications, owing to both underlying disease as well as the intensive chemotherapeutic treatment. Additionally, a significantly higher grade of mucositis was found among cases. However, an independent association between mucositis and increased risk of VRE acquisition, as established by others previously, could not be confirmed by multivariate analysis.[17] One explanation of the association between mucositis and VRE acquisition might be that diffuse gastrointestinal

mucosal breakdown promotes the likelihood of colonization of the gut with VRE. Subsequently, growth of VRE to high numbers may lead to bacteraemia. In line with this hypothesis, we found a greater frequency of diarrhoea and faecal incontinence in our VRE-positive population. Like mucositis, diarrhoea may serve as a parameter of changes in gastrointestinal tract function, increasing the likelihood of colonization with VRE. This concept has been proposed previously in burn patients.[7]

Table 2. Variablese entered in the multivariate model

Potential risk factor	Cases (n= 24)	Controls (n= 49)	OR ($CI_{95\%}$)	*P*-value
AML	11 (46)	4 (8)	6.1 (0.6-58.8)	NS
Antibiotics within 1 month before admission	15 (63)	6 (12)	13.0 (2.1-80.5)	0.006
Diarrhoea	17 (71)	10 (20)	3.4 (0.6-19.0)	NS
Days on ward before outbreak (days ± SD)	25.8 ± 28.1	10.1 ± 21.0	1.0 (0.9-1.1)	NS
Mucositis (mean grade ± SD)	1.1 ± 0.9	0.6 ± 0.8	1.7 (0.7-4.6)	NS
Albumin level at baseline (mean, g/L)	33.2 ± 5.9	37.8 ± 6.5	1.2 (1.1-1.3)	0.02
Fever (t > 38.5 ºC)	19 (79)	18 (37)	0.6 (0.1-3.8)	NS

Values represent the number (%) of patients unless otherwise indicated. SD, standard deviation: NS, not significant.

Multivariate analysis of our data revealed that antibiotic use within 1 month before admission was independently associated with VRE colonization or infection. Prior administration of antibiotics has been consistently recognized as an important risk factor for the acquisition of VRE, probably because the intensive use of antimicrobial agents provides VRE with a selective growth advantage. Previous case-control studies have implicated vancomycin, [18] third-generation cephalosporins[6,18] and prior exposure to antibiotics with activity against anaerobes[4,19] as significant risk factors. In view of these data, the adjustment of our empirical antibiotic regimen, replacing cefpirome with imipenem-cilastatin, might be debated, because the latter possesses anti-anaerobic effects.

A second risk factor independently associated with VRE acquisition in our multivariate model was the albumin value at baseline, which was significantly lower for cases. It is likely that the albumin concentration is a highly sensitive indicator of preclinical disease and disease severity.[20] Moreover, in various populations of patients, a low albumin value on admission has been identified as an independent risk factor for colonization or infection with several microorganisms, including respiratory-tract pathogens, methicillin-resistant *Staphylococcus aureus* (MRSA) and yeasts.[21-23]

A total of five cases developed VRE bloodstream infections. One of the major risk factors for the development of invasive VRE infection appears to be the presence of gastrointestinal colonization.[4] In our study, prior colonization with VRE was established in both patients who developed VRE bacteraemia during the period that patients were systematically screened for colonization. Until recently, no effective antimicrobial agents were available for treating these patients. We administered quinupristin/dalfopristin (Synercid®), a new streptogramin antibiotic, to four patients with proven VRE bacteraemia (three cases and one patient with a non-outbreak VRE).[24] The clinical success rate was excellent and all patients became culture negative. Three patients survived the neutropenic episode, one patient died of respiratory failure, without signs of active VRE infection.

Our infection control measures were taken according to the Centers for Disease Control (CDC) recommendations for the prevention of the spread of vancomycin resistance,[25] focusing mainly on early detection of VRE carriage, education of hospital staff and a critical consideration of the standard antibiotic regimen. We restricted the use of vancomycin and banned all cephalosporins. Strict hand disinfections with an alcoholic hand rub by all personnel and patients was reinforced. In addition to these general measures, some specific interventions may have played a key role in the prevention of further spread of VRE.

First, in addition to the cohorting of patients, nurses were strictly allocated to either VRE-positive or -negative patients. Some of our newly admitted patients became VRE positive even while being nursed in strict isolation in a single room or in one of the controlled airflow rooms. It appeared likely that VRE was transmitted via the attending staff, although we did not perform systematic surveillance cultures in medical personnel to prove this observation. The transmission of VRE from the hands of healthcare workers to patients appears to play a major role in the nosocomial spread of the microorganism and exposure to a nurse caring for another VRE-positive patient has been identified as a significant risk factor for the acquisition of VRE.[3,4] Mathematical analysis of transmission dynamics of VRE has shown that hand washing and allocation of staff are the most powerful infection control measures in endemic settings.[26]

Second, we learned not to rely on negative surveillance culture results in patients who could have acquired the epidemic VRE during previous admissions. Several patients who had stayed on the ward before and even patients who were known to be VRE positive, had VRE-negative surveillance cultures at their re-admission, but subsequently proved to carry the epidemic VRE. This finding led to the definition of a 'possibly VRE-positive' cohort. We suggest that the number of VRE in the bowel decreases below detection levels in periods that

the patient is not treated with antimicrobial agents. When appropriate selective antimicrobial pressure is enforced, VRE numbers may increase accordingly. It has been shown that colonization with the same strain of VRE may persist for at least a year.[27]

Third, a measure thought to be essential in the control of the outbreak was the allocation of private portable toilet facilities to every single patient. Our ward was constructed about 35 years ago and patients had to share a limited number of toilet facilities (two for women, two for men, shared by 18 patients), located outside the patient rooms. This might have contributed to the initial spread of VRE. The toilet has been clearly identified as a transmission route for VRE.[28]

Until recently, major differences in the epidemiology of VRE infections existed between Europe and the United States. VRE seems to have become endemic in American hospital populations and many nosocomial outbreaks of VRE have been reported.[2-4,7] To date, the occurrence of nosocomial infections with VRE in Europe has been rare. However, the increasing number of reports on VRE outbreaks in Europe and outside the United States may indicate that a change in epidemiology of VRE towards the American situation is ahead. Given the threat posed by VRE in hospitals worldwide, much is to be gained by a proper understanding of issues involving nosocomial VRE infections, especially by physicians involved in the care of immunocompromised patients.

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Chapter 9

Summary and concluding remarks

Summary and conclucing remarks

In the Netherlands approximately 7000 patients are diagnosed with a malignancy of the blood or bone marrow every year. Prospects for these patients have substantially improved over the past decades. The field of clinical haematology is characterized by rapid and sometimes exciting innovations in treatment options, achieving higher remission rates and control over diseases that were deemed incurable until recently. The advent of combination chemotherapy, dose intensification, autologous and allogeneic stem cell transplantation and immunotherapy all have contributed substantially to this progress in the treatment of haematological malignant diseases. Unfortunately, these important and promising developments are counterbalanced by serious impairment of host immune response mechanisms. This occurs in a population of patients that is already running a serious risk of infections, because haematological malignancies reside by definition in the immune system itself. As a consequence, these patients are highly susceptible to infections with various kinds of opportunistic pathogens, including viruses, bacteria and fungi.

Infections in patients with aggressive haematological malignancies are associated with significant morbidity and mortality. Moreover, their initial presentation frequently is erratic, with sometimes only minimal signs and symptoms. Therefore, it is not surprising that anti-infective strategies have focused on prevention and early treatment. Antibacterial and antifungal prophylaxis and early, or 'empirical' antibiotic therapy have gained a lot of interest in the international literature. In **chapter 2** an overview is given on current insights and developments on antibacterial and antifungal prophylaxis and treatment, from a clinical perspective.

In view of the serious consequences of infections in patients treated for haematological disease, it is tempting to clinicians to readily introduce newly marketed and promising antibacterial and antifungal drugs into daily clinical practice, before results of relevant clinical trials have become available. This entails the risk of neglecting important questions considering safety, pharmacokinetic behaviour and efficacy of the drug involved, as prophylaxis or treatment in the specific population of patients with haematological diseases.

The studies described in this thesis aimed to explore the safety and efficacy of a selection of new antimicrobials, for the prevention of bacterial (**chapter 3 and 4**) and fungal (**chapter 5**

and 6) infections, as well as for the empirical treatment of bacterial infections (**chapter 7**). The occurrence of an outbreak of vancomycin-resistant *Enterococcus faecium* (VRE) led to an assessment of risk factors for the acquisition of VRE and recommendations for the prevention and control of such an outbreak (**chapter 8**).

Main results

Chapter 3. Gram-positive breakthrough infections pose a major drawback to the use of quinolones for antibacterial prophylaxis in neutropenic patients. Levofloxacin, a relatively new quinolone, has an augmented Gram-positive spectrum and may potentially overcome this problem. When administered orally (500 mg, once daily) as antibacterial prophylaxis to patients receiving intensive chemotherapy for haematological malignancies, levofloxacin provided adequate eradication of Gram-negative microorganisms and *S. aureus* and preserved the anaerobic component of the bowel flora. It was found that the pharmacokinetic properties of levofloxacin were not altered during the phase of chemotherapy and neutropenia. Minimal inhibitory concentration (MIC) values for viridans group (VG) streptococci tended to increase, which cautioned against the occurrence of acquired resistance to levofloxacin.

Chapter 4. Elaborating the results described in chapter 3, the question remained open as to how levofloxacin prophylaxis compared with standard prophylactic regimens, considering efficacy, tolerability and induction of resistance among bacterial pathogens. In an attempt to answer this question, levofloxacin was compared with ciprofloxacin plus phenethicillin as antibacterial prophylaxis during neutropenia, in a randomized clinical trial. It was found that levofloxacin and ciprofloxacin-phenethicillin were equally effective in the prevention of bacterial infections in neutropenic patients. However, levofloxacin was better tolerated, which may benefit compliance with therapy. In line with the findings described in chapter 3, resistance to levofloxacin was observed among VG streptococci, but timely adjustments of the prophylactic regimen were made, based on surveillance culture data and no break-through infections with VG streptococci occurred.

Chapter 5. In an attempt to improve prophylaxis against the occurrence of invasive fungal infections in neutropenic patients, a randomized clinical trial was designed to compare a lipid formulation of amphotericin B intravenously (amphotericin B colloidal dispersion, ABCD)

with oral fluconazole. However, the administration of ABCD for prophylactic reasons to patients without life-threatening fungal infections, was associated with major and intolerable side-effects during infusion of the compound. The study was prematurely terminated and it was concluded that ABCD appeared to be unsuitable for antifungal prophylaxis in neutropenic patients.

Chapter 6. It is well documented that administration of itraconazole increases cyclosporin A (CsA) serum concentrations. However, there are no data on the effects of CsA on itraconazole pharmacokinetics. Itraconazole and hydroxy-itraconazole pharmacokinetic parameters were studied, before and during administration of cyclosporin A (CsA) in ten patients receiving an allogeneic stem cell transplantation. It was found that exposure to OH-itraconazole, but not to itraconazole is increased when itraconazole is co-administered with CsA. Monitoring of itraconazole serum concentrations is important in patients who use drugs that, like itraconazole, interact with CYP3A4 and when target drug concentrations are to be achieved for optimal antifungal efficacy.

Chapter 7. With the introduction of cefpirome, a new fourth generation cephalosporin, as empirical treatment of febrile neutropenia, a cohort study was conducted to assess the clinical efficacy of cefpirome and its activity against isolated pathogens. Fifty-three percent of patients survived the neutropenic episode without the need of treatment modification (success-rate 53%). Susceptibility testing of isolated pathogens showed adequate coverage of a broad range of Gram-positive and Gram-negative microorganisms including viridans group streptococci, coagulase-negative staphylococci, *Enterobacteriaceae* and *Pseudomonas aeruginosa*. In addition, pharmacokinetic data indicated that a dosing regimen of cefpirome 2 g twice daily was sufficient in this population.

Chapter 8. This chapter describes the occurrence of an outbreak of vancomycin-resistant *Enterococcus faecium* (VRE) on our haematology ward. A case-control study showed that cases (n = 24) had a longer stay on the ward during the year preceding the outbreak, as compared with VRE-negative control patients (n = 49). More cases had acute myeloid leukaemia and cases had higher grades of mucositis. Logistic regression analysis identified antibiotic use within 1 month before admission and low albumin levels at baseline to be independent risk factors for acquisition of VRE. Control of the outbreak was achieved by a

step-wise implementation of intensive infection control measures, which included the cohorting of patients, allocation of nurses and reinforcement of hand hygiene.

Conclusions and clinical implications

The studies described in this thesis have originated from questions and problems that were encountered in daily clinical practice, during the care for patients with haematological malignancies. From the results reported here, some answers to these questions can be deduced, which may contribute to actual and future decisions with regard to the prevention and treatment of infections in these patients:

[1] Levofloxacin may be considered as standard treatment for the prevention of bacterial infections in neutropenic patients, considering its good tolerability and equal efficacy as compared with ciprofloxacin-phenethicillin. Surveillance cultures are mandatory, to closely monitor the emergence of levofloxacin-resistant VG streptococci and adjustments of the prophylactic regimen must be made accordingly.
[2] Amphotericin B colloidal dispersion (ABCD) is not suitable for antifungal prophylaxis in neutropenic patients with a haematological malignancy, due to excessive infusion-related toxicity. If used for therapeutic indications, close pursuit of adverse events is strongly advised.
[3] Exposure to OH-itraconazole may be increased when itraconazole is co-administered with Cyclosporin A. This finding is of limited clinical relevance, but may be may be important in the occasional event that monitoring of serum itraconazole concentrations is warranted.
[4] The use of cefpirome as empirical antibacterial treatment in patients with febrile neutropenia can not be strongly recommended based on the data presented in this thesis. Though we found a rather good efficacy, there are some points of concern; (a). Limited activity against *Pseudomonas aeruginosa* may in the long run hamper the use of cefpirome as single agent therapy. (b). The use of cefpirome was thought to be associated with the occurrence of an outbreak of vancomycin resistant *Enterococcus faecium,* though chapter 7 nor chapter 8 provide data to fully support this assumption. (c). In a recently published meta analysis on empirical antibiotic monotherapy for febrile neutropenia, cefepime, which closely resembles cefpirome, was associated with a higher mortality rate than other antibiotics, probably due to less efficacy.[1]

[5] Antibiotic use within 1 month before admission and low albumin levels at baseline are independent risk factors for acquisition of vancomycin-resistant *Enterococcus faecium*, during a nosocomial outbreak. Control of the outbreak is achievable, by intensive infection control measures.

Future directions

There is now growing evidence that both prophylactic and empirical administration of antibacterial and antifungal antibiotics may reduce mortality and morbidity among patients with severe neutropenia. The data presented in this thesis, however, are too limited to add to the existing evidence on those issues. Our findings rather illustrate the reverse side of the medal, which displays the concerning emergence of resistant pathogens and increased toxicity after the introduction of new prophylactic or therapeutic antibiotics. The large number of reports on anti-microbial resistance and nosocomial outbreaks on haematology or oncology wards, following the application of new prophylactic or therapeutic strategies and to which this thesis adds, can not be neglected.[2-9] Here, not only the well-being of the individual patient is at risk, but also of the population at large. Moreover, increased toxicity of antibiotics and the emergence of resistant microorganisms may have considerable effects on daily care and management of haemato-oncology units and may substantially increase work load for medical staff and health-care costs.
Although these concerns argue against the widespread and unlimited use of antibiotics in patients with neutropenia, a balanced appraisal is needed, that should give direction to future research.

[1] It is noteworthy that the emergence of resistant strains not necessarily leads to subsequent infection with the microorganism involved.[10-12] The reduction in mortality and infection rates appears to outweigh the detriments of emerging resistant microorganisms. Future studies should not only focus on the development of resistance among potential pathogens, but also on the probability and severity of subsequent invasive infections.

[2] One of the major limitations of the studies described in this thesis is the lack of subdivision of the population of patients as a whole, into different categories of risk levels for infectious complications. For example, patients who are treated with an autologous stem cell

transplantation for Non Hodgkin's lymphoma are at a different risk and will acquire other types of infections than patients who receive an allogeneic stem cell transplantation for acute leukaemia. To date, efforts are made to distinguish categories of risk of infections among patients with neutropenia.[13-15] Future studies should aim to identify the population of patients that is likely to benefit from a given agent the most.[16-18] In selected groups of low-risk patients therapy may be simplified or even discontinued.

[3] Future studies should focus on the development and improvement of sensitive and rapid diagnostic techniques, to trace infectious complications in neutropenic patients. Early detection of bacterial or fungal infections and accurate identification of the pathogens involved, may lead to earlier and more appropriate antibiotic intervention, thereby increasing the success of therapy. In selected groups of patients, improved diagnostic procedures and effective early treatment may even replace prophylaxis and in potential, will reduce the empirical overtreatment of patients with persisting fever during the neutropenic episode.

So, rather than refraining from the routine use of antibiotics in patients with neutropenia, the clinician faces a challenge to chose the right antibiotic regimen for the right population of patients. Not only data from clinical trials should guide these decisions. Other factors, that are at least as important, include local bacteriological and epidemiological data, with an emphasis on resistance patterns of predominantly isolated microorganisms, as well as the utility of an antibiotic in daily practice, its user-friendliness to patients and nursing staff, its toxicity and costs.

Nederlandstalige samenvatting en conclusies

In Nederland wordt elk jaar bij ongeveer 7000 patiënten een kwaadaardige aandoening van bloed of beenmerg vastgesteld. De vooruitzichten voor deze patiënten zijn de laatste jaren sterk verbeterd. Het vakgebied van de klinische hematologie wordt gekenmerkt door snelle en vaak spectaculaire ontwikkelingen in de behandelingsmogelijkheden, waardoor hogere genezingspercentages worden bereikt en patienten kunnen worden behandeld met ziektes die tot recent daarvoor niet in aanmerking kwamen. De toepassing van combinatiechemotherapie, het intensiveren van de dosis, autologe en allogene stamceltransplantatie en immunotherapie hebben allen in belangrijke mate bijgedragen aan de vooruitgang van de behandelingsmogelijkheden in de hemato-oncologie. Een belangrijk nadeel is echter dat deze op zich veelbelovende ontwikkelingen, zonder uitzondering, het afweersysteem sterk in negatieve zin beïnvloeden. Bovendien gaat het daarbij om een categorie patiënten die al op voorhand een verhoogde kans op infecties heeft, omdat een hemato-oncologische ziekte per definitie zijn oorsprong vindt in het immuunsysteem zelf. Dit alles heeft tot gevolg dat deze patiënten zeer gevoelig zijn voor infecties met een scala aan opportunistische micro-organismen, waaronder diverse soorten virussen, bacteriën en schimmels.

Infecties bij patiënten met hematologische maligniteiten kunnen leiden tot ernstige ziekteverschijnselen en ook sterfte. De eerste presentatie van een dergelijke infectie kan echter aspecifiek zijn, met soms minimale ziekteverschijnselen. Het is dan ook niet verwonderlijk dat strategieën ter bestrijding van deze infecties vooral gericht zijn op preventie en vroege behandeling. Medicamenteuze profylaxe tegen bacteriën en schimmels en vroeg ingestelde, zogenaamde ‘empirische’ therapie staan sterk in de belangstelling in de internationale literatuur. In **hoofdstuk 2** van dit proefschrift wordt vanuit een klinisch perspectief een overzicht gegeven van de huidige inzichten en ontwikkelingen op het gebied van antibacteriële en antifungale profylaxe en behandeling.

Gezien de ernstige gevolgen van infecties voor patiënten die behandeld worden voor een hemato-oncologische ziekte, hebben behandelend artsen vaak de neiging om veelbelovende medicamenten, soms net op de markt, snel te introduceren in de dagelijkse praktijk. Dit gebeurt nogal eens voordat kan worden beschikt over duidelijke gegevens uit klinisch onderzoek. Daarmee blijven belangrijke vragen onbeantwoord met betrekking tot de veiligheid van een medicament, het farmacologisch gedrag en de werkzaamheid van het

betreffende middel als profylaxe of als behandeling, in de bijzondere populatie van patiënten met hematologische ziekten.

De studies beschreven in dit proefschrift hebben tot doel de veiligheid en werkzaamheid te onderzoeken van een selectie van nieuwe antibiotica, ingezet voor de preventie van bacteriële- (**hoofdstuk 3 en 4**) en schimmelinfecties (**hoofdstuk 5 en 6**) en voor de empirische behandeling van bacteriële infecties (**hoofdstuk 7**). Een uitbraak met vancomycine-resistente *Entrococcus faecium* (VRE) gaf aanleiding tot een systematische inventarisatie van risicofactoren voor besmetting met VRE en leidde tot aanbevelingen voor het voorkomen en bestrijden van een dergelijke uitbraak (**hoofdstuk 8**).

Belangrijkste resultaten

Hoofdstuk 3. Het optreden van doorbraakinfecties met Gram-positieve bacteriën vormt een belangrijk nadeel van het gebruik van quinolonen (antibiotica) als profylaxe bij neutropene patiënten. Levofloxacin is een relatief nieuw quinolone antibioticum met een betere antibiotische werking tegen Gram-positieve bacteriën dan eerdere quinolonen en zou daardoor een oplossing kunnen bieden voor dit probleem. Toediening van levofloxacin (éénmaal daags 500 mg, oraal) als antibacteriële profylaxe, aan patiënten die behandeld werden met intensieve chemotherapie voor een hematologische maligniteit, resulteerde in een adequate eradicatie van het Gram-negatieve deel van de darmflora en van *Staphylococcus aureus*, waarbij het anaërobe deel van de darmflora in tact bleef. Daarbij kon worden aangetoond dat de farmacokinetische eigenschappen van levofloxacin niet veranderden gedurende de periode van toediening van de chemotherapie en de neutropene fase. De waarden van de minimaal remmende concentratie (MIC) voor viridans streptokokken bleek geleidelijk te stijgen, hetgeen wijst op het optreden van resistentie tegen levofloxacin.

Hoofdstuk 4. Met de resultaten zoals beschreven in hoofdstuk 3, bleef de vraag onbeantwoord hoe levofloxacin zich verhoudt tot de huidige antibacteriële profylaxe, vooral wat betreft werkzaamheid, bijwerkingen en tolerantie en het optreden van resistentie bij bacteriën. Om deze vraag te beantwoorden werd een klinische gerandomiseerde studie verricht, waarbij levofloxacin werd vergeleken met ciprofloxacin plus phenethicilline als antibacteriële profylaxe gedurende neutropenie. Beide middelen bleken even effectief in het

voorkomen van bacteriële infecties bij neutropene patiënten. Levofloxacin werd echter beter verdragen, hetgeen gunstig zal zijn voor de therapietrouw. Zoals ook beschreven in hoofdstuk 3, bleken viridans streptokokken soms resistentie te ontwikkelen tegen levofloxacin. Op basis van de resultaten van inventarisatiekweken kon in die gevallen het profylactische antibiotica schema tijdig worden aangepast en doorbraakinfecties met viridans streptokokken werden niet waargenomen.

Hoofdstuk 5. In een poging om de profylaxe tegen schimmelinfecties te verbeteren werd een gerandomiseerde klinische studie ontworpen, om intraveneuze toediening van een lipide-vorm van amfotericine B (amfotericine B in colloïdale dispersie, ABCD) te vergelijken met fluconazol oraal. De toediening van ABCD als profylaxe, aan patiënten die geen levensbedreigende schimmelinfecties hadden, bleek echter gepaard te gaan met ernstige en onacceptabele bijwerkingen tijdens de infusie van het middel. De studie werd dan ook voortijdig gestaakt en geconcludeerd werd dat ABCD niet geschikt is als antifungale profylaxe bij neutropene patiënten.

Hoofdstuk 6. Het is een bekend gegeven dat toediening van itraconazol de bloedspiegel van ciclosporine (cyclosporine A) verhoogt. Er zijn echter geen gegevens over het effect van ciclosporine op de farmacokinetiek van itraconazol. De farmacokinetische eigenschappen van itraconazol en hydroxy (OH) itraconazol werden bestudeerd vóór en tijdens toediening van ciclosporine, bij tien patiënten die een allogene stamcel transplantatie ondergingen. De blootstelling aan OH-itraconazol bleek significant te zijn toegenomen wanneer itraconazol gelijktijdig werd toegediend met ciclosporine, dit bleek niet het geval te zijn voor de blootstelling aan itraconazol zelf. Het meten van itraconazol spiegels kan van belang zijn als patiënten daarnaast andere medicamenten gebruiken, die net als itraconazol worden afgebroken via het cytochroom P450 (CYP) 3A enzym systeem. Dit is vooral van toepassing wanneer bepaalde concentraties van dit medicament moeten worden behaald om een optimaal antischimmel effect te verkrijgen.

Hoofdstuk 7. De introductie van cefpirom, een destijds nieuw vierde-generatie cefalosporine, voor de empirische behandeling van neutropene patiënten met koorts, op de afdeling hematologie van het VU medisch centrum, leidde tot de uitvoering van een klinisch cohort onderzoek om de effectiviteit van cefpirom te onderzoeken. Ook werd de werkzaamheid tegen de meest geïsoleerde ziekteverwekkers onderzocht. Drieënvijftig procent van de patiënten

overleefde de neutropene episode zonder dat wijziging van antibiotica nodig was (succespercentage 53%). Gevoeligheidsbepalingen bij geïsoleerde bacteriën toonde aan dat een breed scala aan Gram-positieve en Gram-negatieve bacteriën gevoelig was voor cefpirom, met name viridans streptokokken, coagulase-negatieve stafylokokken, *Enterobacteriae* en *Pseudomonas aeruginosa*. Daarbij liet farmacokinetisch onderzoek zien dat toediening van twee maal daags 2 gram cefpirom afdoende zou moeten zijn in deze populatie.

Hoofdstuk 8. Dit hoofdstuk beschrijft een uitbraak van vancomycine-resistente *Enterococcus faecium* (VRE) op de afdeling hematologie van het VU medisch centrum. Een patiënt-controle onderzoek toonde aan dat besmette patiënten (n = 24) langer opgenomen waren geweest op de afdeling gedurende het jaar voorafgaand aan de uitbraak in vergelijking met VRE-negatieve controle patiënten (n = 49). Onder de besmette patiënten waren meer gevallen van acute myeloide leukemie en in deze groep was ook de mate van slijmvliesbeschadiging door chemotherapie (mucositis) hoger dan in de controlegroep. Door middel van logistische regressie analyse kon worden aangetoond dat antibiotica gebruik in de maand voorafgaand aan de opname en een lage albuminewaarde bij aanvang onafhankelijke risicofactoren waren voor besmetting met VRE. De uitbraak kon succesvol worden bestreden door een stapsgewijze inzet van intensieve infectie beperkende maatregelen, waaronder het indelen van de patiëntenpopulatie in drie groepen (besmet, onbesmet of verdacht), het toewijzen van specifieke verpleegkundigen aan zo'n categorie en het verplichten van betere handhygiëne.

Conclusies en klinische toepasbaarheid

De studies beschreven in dit proefschrift zijn gebaseerd op vraagstukken en problemen die voortkwamen uit de dagelijkse klinische praktijk, tijdens de zorg voor patiënten met een hemato-oncologische ziekte. Uit de in dit proefschrift beschreven resultaten van deze studies kunnen antwoorden op deze vragen worden afgeleid, die richting kunnen geven aan actuele en toekomstige beslissingen ten aanzien van preventie en behandeling van infecties bij deze patiënten.

[1] Het gebruik van levofloxacin als standaard antibacteriële profylaxe bij neutropene patiënten is het overwegen waard, gezien het feit dat het middel uitstekend verdragen wordt en de werkzaamheid vergelijkbaar is met die van ciprofloxacin en phenethicilline. Het

verrichten van inventarisatiekweken is daarbij een vereiste, om het optreden van levofloxacin-resistente viridans streptokokken te signaleren en het antibacteriële profylactische schema tijdig te kunnen aanpassen.

[2] Amfotericine B in colloïdale dispersie (ABCD) is niet geschikt voor de preventie van schimmelinfecties bij neutropene patiënten met een hematologische maligniteit vanwege ernstige, infusiegerelateerde bijwerkingen. Indien dit middel gebruikt wordt voor therapeutische doeleinden is het nauwkeurig observeren van bijwerkingen sterk aan te raden.

[3] Blootstelling aan OH-itraconazol kan verhoogd zijn als itraconazol en ciclosporine gelijktijdig worden toegediend. Deze bevinding heeft beperkte klinische betekenis, maar kan van belang zijn als het meten van itraconazol spiegels is aangewezen.

[4] De toepassing van cefpirom als empirische antibacteriële therapie bij de behandeling van neutropene koorts kan alleen op basis van de gegevens beschreven in dit proefschrift niet zonder meer worden aanbevolen. Hoewel een redelijke effectiviteit van het middel kon worden aangetoond zijn er een aantal zaken die de aandacht behoeven; (a). Een beperkte werkzaamheid tegen *Pseudomonas aeruginosa* is waarschijnlijk een belangrijk nadeel van het gebruik van cefpirom als monotherapie. (b). Het gebruik van cefpirom heeft mogelijk een rol gespeeld bij het optreden van de uitbraak met vancomycine-resistente *Enterococcus faecium*, hoewel hoofdstuk 7 en 8 geen gegevens bevatten die deze aanname kunnen ondersteunen. (c) In een recent gepubliceerde meta-analyse van de toepassing van monotherapie op empirische gronden bij neutropene koorts wordt gemeld dat het gebruik van cefepime, een cefalosporine dat sterke gelijkenis vertoont met cefpirom, gepaard gaat met een hoger sterftepercentage dan andere antibiotica. Dit is waarschijnlijk het gevolg van een mindere werkzaamheid dan de andere geteste antibiotica.[1]

[5] Het gebruik van antibiotica binnen één maand voor opname en een lage albumine waarde bij aanvang van de behandeling van een hemato-oncologische ziekte zijn onafhankelijke risicofactoren voor de besmetting met vancomycine-resistente *Enterococcus faecium* gedurende een ziekenhuisuitbraak. Een dergelijke uitbraak is te bestrijden door toepassing van intensieve infectie beperkende maatregelen.

Toekomstperspectief

Er is toenemend wetenschappelijk bewijs dat de toepassing van profylactische en therapeutische antibacteriële en antifungale antibiotica daadwerkelijk leidt tot een reductie

van ziekteverschijnselen en ook sterfte bij patiënten met ernstige neutropenie. De gegevens beschreven in dit proefschrift zijn echter te beperkt om een belangrijke bijdrage te kunnen leveren aan de bewijsvoering van deze bewering. Onze gegevens illustreren eerder de keerzijde van de medaille, die gekenmerkt wordt door een zorgwekkende toename van resistente bacteriën en het optreden van ernstige bijwerkingen als nieuwe profylactische of therapeutische antibiotica worden ingezet. De grote hoeveelheid wetenschappelijke artikelen gewijd aan ziekenhuisuitbraken op hematologie- of oncologieafdelingen en aan bacteriële resistentie tegen antibiotica illustreert dit nog eens extra.[2-9] Het gaat daarbij niet langer en alleen om het welzijn van de individuele patiënt maar ook van diens directe omgeving en uiteindelijk van de bevolking in zijn geheel. Daarbij komt dat het optreden van ernstige bijwerkingen van antibiotica en de toename van resistente micro-organismen aanzienlijke gevolgen hebben voor de dagelijkse zorg en werkzaamheden op hemato-oncologie afdelingen. Uiteindelijk leidt dit alles tot een verminderde genezingskans voor de patiënt, een toename van de werklast voor medisch personeel en tot stijging van kosten van de gezondheidszorg.
Hoewel deze argumenten pleiten tegen een wijdverbreid en ongelimiteerd gebruik van antibiotica bij neutropene patiënten is een afgewogen oordeel op zijn plaats, hetgeen ook richting kan geven aan onderwerpen van toekomstig onderzoek.

[1] Uit literatuurgegevens en uit de studies beschreven in dit proefschrift blijkt dat het optreden van resistentie niet noodzakelijkerwijs wordt gevolgd door een infectie met het betreffende micro-organisme.[10-12] De afname van sterfte en infectiekans lijken ruimschoots op te wegen tegen de nadelen die de aanwezigheid van resistente micro-organismen met zich meebrengt. Toekomstig onderzoek zou zich naast onderzoek naar resistentiepatronen, ook dienen te richten op de kans dat daadwerkelijk een infectie met het betreffende micro-organisme volgt, met een beoordeling van de ernst daarvan.

[2] Eén van de belangrijkste beperkingen van de studies beschreven in dit proefschrift is het ontbreken van een indeling van de patientenpopulatie in verschillende categorieën, naar het risico op het optreden van infecties. Patiënten die bijvoorbeeld worden behandeld met een autologe stamcel transplantatie voor een Non-Hodgkin lymfoom lopen een ander risico op infecties, met andersoortige verwekkers, dan patiënten die een allogene stamcel transplantatie ondergaan vanwege acute leukemie. Tegenwoordig worden pogingen gedaan om een dergelijke risicoindeling te maken.[13-15] Toekomstig onderzoek zou ten doel moeten hebben om juist die populatie te identificeren die het meeste baat heeft bij toediening van een bepaald

medicament.[16-18] In geselecteerde groepen van patiënten met een laag infectierisico, is het denkbaar dat het voorschrijven van antibiotica sterk wordt teruggebracht of zelfs geheel achterwege wordt gelaten.

[3] Toekomstig onderzoek zou zich dienen te richten op de ontwikkeling en verbetering van diagnostische technieken om infecties bij patiënten met neutropenie op te sporen. Vroegtijdige ontdekking van een bacteriële- of schimmelinfectie en identificatie van het betrokken micro-organisme kan bijdragen aan een snellere en beter afgestemde behandeling, waardoor het succes daarvan zal toenemen. In geselecteerde patiëntengroepen kunnen verbeterde diagnostische procedures, zo nodig gevolgd door tijdig ingestelde therapie, wellicht het profylactisch gebruik van antibiotica overbodig maken en de overbehandeling met antibiotica op empirische basis reduceren.

Dus, in plaats van volledig af te zien van het routinematig gebruik van antibiotica bij patiënten met een hematologische maligniteit, ziet de behandelend arts zich gesteld voor de uitdaging om het juiste antibioticum voor te schrijven aan de juiste categorie patiënten. Niet alleen gegevens uit klinisch onderzoek zijn daarbij van belang. Andere factoren, die minstens zo belangrijk zijn bij deze beslissing, zijn bijvoorbeeld plaatselijke microbiologische en epidemiologische gegevens. Daarbij dient gelet te worden op resistentiepatronen van micro-organismen die in het betreffende centrum frequent worden geïsoleerd. Ook het gebruiksgemak van een antibioticum is van belang, zowel voor patiënten als voor verpleegkundigen. Tenslotte zal de keuze voor een bepaald middel worden bepaald door de bijwerkingen van het middel en de kosten.

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Dankwoord

Het verrichten van patiëntgebonden klinisch onderzoek is een stuk eenvoudiger als de onderzoeker werkzaam is in het instituut waar die patiënten zich ook feitelijk bevinden. Omdat ik het grootste deel van mijn dagen doorbreng in een ziekenhuis ongeveer 5 kilometer bezuiden het VUMC, ben ik veel dank verschuldigd aan hen die, zowel geografisch als inhoudelijk dichtbij het onderzoeksterrein, bereid waren zich in te zetten. Hun bijdrage en hulp zijn onmisbaar geweest bij het totstandkomen van dit proefschrift. Een aantal van deze personen wil ik hier met name noemen.

Prof. dr. P.C. Huijgens, beste Peter, jouw in mijn co-assistententijd (met licht Amsterdams accent) uitgesproken woorden "seg Timmers, wat wil jij eigenlijk in het leven?", hebben richting gegeven aan mijn verdere ontwikkeling en loopbaan als arts. Jij gaf mij de gelegenheid om hematoloog te worden, binnen een groep mensen voor wie maximale en professionele inzet voor elke individuele patiënt hand in hand gaan met humor en relativeringsvermogen. Naar dat voorbeeld wil ik tot op de dag van vandaag werken. Dank voor je vertrouwen en de kansen die je me hebt gegeven om dit proefschrift in eigen tempo af te ronden.

Mw. dr. A.M. Simoons-Smit, beste Ina, van het begin tot het einde heb jij me bijgestaan bij het vormgeven van wat uiteindelijk dit proefschrift is geworden. Zonder jouw inhoudelijke sturing van de verschillende onderwerpen, en daarna je altijd nauwkeurige en heldere commentaar op de artikelen was dit zeker niet in zijn huidige vorm tot stand gekomen. In duistere dalen, waar het pad der wetenschap kennelijk al te vaak doorheen leidt, wist jij me steeds weer te motiveren of de helpende hand te bieden. Ik dank je zeer en verheug me op het voortzetten van onze samenwerking, binnen het toekomstige samenwerkingsverband tussen de afdeling Medische Microbiologie van het VUMC en Ziekenhuis Amstelland.

Prof. dr. G.J. Ossenkoppele, beste Gert, jij bent voor mijn vorming tot hematoloog van cruciale betekenis geweest. Van jou leerde ik over 'paprikapoeder in sigarettenrook', van 'billocyten' en nog veel meer. Tot op de dag van vandaag is het een genoegen om met je te mogen overleggen in de consultatieve sfeer. Ik hecht sterk aan je heldere en altijd overwogen adviezen, vaak compleet met een uit het hoofd geciteerd relevant artikel (met tijdschrift en jaargang). Dat jij wel het Elfstedenkruis (zelfs meerdere) bezit, terwijl ik 't moet doen met een

'speldje' van de Weissensee voegt alleen maar toe aan mijn respect. Dank voor je bereidheid om in de promotiecommissie zitting te nemen.

Prof. dr. C.M.J.E. Vandenbroucke-Grauls, beste Christina, het verrichten van wetenschap betekende voor mij vooral nog veel te moeten leren. Ik heb veel van je geleerd met name op het methodologisch en epidemiologisch vlak. Zonder jouw inbreng en zonder de menskracht en middelen van jouw afdeling was de uitvoering van de meeste studies in dit proefschrift niet mogelijk geweest. Dank daarvoor en ook voor je bereidheid om te opponeren tijdens de promotie.

De overige leden van de promotiecommissie, prof. dr. J.H. Beijnen, dr. A.W. Dekker, drs. A.C. van Loenen, prof. dr. M.H.J. van Oers en zijn vervanger in de oppositie dr. B.J. Biemond, wil ik danken voor hun bereidheid om aandacht te besteden aan mijn proefschrift en om zitting te nemen in de promotiecommissie.

De stafleden van de afdeling hematologie wil ik bedanken voor hun bijdrage aan dit proefschrift. Josée Zijlstra, Sonja Zweegman, Mariëlle van Wondergem, Jeroen Janssen, Otto Visser, Arjan van de Loosdrecht en Roel Jonkhoff, dank voor jullie bereidheid om de klinische studies ook daadwerkelijk tot uitvoering te brengen en de passerende afdelingsartsen daar telkens weer voor te motiveren. Dank voor jullie betrokkenheid en medeleven, ik ben er trots op dat ik destijds deel mocht zijn van het 'team' en voel mij nog steeds nauw met jullie verbonden.

Alle verpleegkundigen van de afdeling hematologie (3b, in mijn tijd 7 west) dank ik voor hun inspanningen om alle pilletjes op tijd uit te delen en keelwatten, poepjes, plasjes en bloedmonsters op tijd te verzamelen. Ik denk nog altijd met veel plezier terug aan mijn tijd op de afdeling.

De hoofden van de apotheek en afdeling klinische farmacologie van het VUMC, drs. A.C. van Loenen en dr. E.L. Swart, beste Arie en Noortje, ik dank jullie voor je inhoudelijke bijdrage aan de artikelen in dit proefschrift en voor alle faciliteiten die door de apotheek ter beschikking van het onderzoek werden gesteld. Veel dank ben ik verschuldigd aan Yvonne Dijstelbloem, Agnes Veldkamp, Bram Wilhelm en Daan Touw, voor hun bijdrage aan de farmacologische inhoud van de studies en voor het feit dat ze geprobeerd hebben mij wegwijs

te maken in het ingewikkelde vakgebied van de klinische farmacologie. Ik wil de medewerkers van het apotheek-laboratorium hartelijk danken voor alle spiegelbepalingen.

De medewerkers van het laboratorium van de medische microbiologie wil ik danken voor al hun inspanningen bij het uitvoeren van de kweken en resistentiebepalingen van al die 'vieze beestjes' die in dit proefschrift de revue passeren. Ook een woord van dank aan dr. P.H.M. Savelkoul, beste Paul, dank voor je tijd en energie die je hebt gestoken in de moleculaire typering van de VRE stammen en de vertaling daarvan, naar ook voor mij begrijpelijke resultaten.

Ik dank de overige medeauteurs van de artikelen voor hun inzet en inspirerende bijdrage. Met name wil ik noemen dr. W.C. van der Zwet, beste Wil, het samen uitpuzzelen van de statistiek bij het 'VRE artikel' was buitengewoon leerzaam voor mij. Drs. D.G. van Vuurden, beste Dannis, dank voor je trouwe inzet bij het cefpirom onderzoek en voor het feit dat je me wegwijs hebt gemaakt in de wereld die 'MS Access' heet. Drs. L.W. Kessels, beste Lonneke, je was deelgenoot van het onderwerp in dit proefschrift dat met afstand de meeste hoofdbrekens heeft gekost, de itraconazole studie, dank voor al je hulp.

Grote waardering heb ik gekregen voor de 'dames van het hematologie trialbureau', Yvonne den Hartog, Marjan Leisink, Cinthy van Roessel, Corien Eeltink, Joke van Wegen en Maaike Leidekker. Ik heb vele uren doorgebracht bij jullie in het hok, achter de PC, om stapels dossiers door te ploegen, altijd op jacht naar 'data'. Ik werd door jullie met vele kopjes koffie op de been gehouden en aan het einde van de dag was ik meestal blij dat ik de volgende dag weer wat anders kon gaan doen. Jullie doen dit werk dagelijks en vol overgave. Een speciaal woord van dank aan Maaike Leidekker, beste Maaike, zonder jouw hulp zou ik de 'levostudie' nooit tot een goed einde hebben kunnen brengen. Dank voor je enorme inzet, je nauwkeurigheid bij het includeren van patiënten en de hulp bij het verzamelen van alle gegevens.

De secretaresses van de afdeling hematologie Annette Bredero, Maartje Ens en Marjan Jalink, dank ik voor hun bereidheid briefjes te plakken op piepers, promotors op te sporen, dossiers op te vragen en verder voor alle hand- en spandiensten verricht ten bate van mijn proefschrift.

De groep vrienden die ik mijn maatschapsleden mag noemen, dr. C. Rustemeijer, dr. C van Groeningen, dr. L. A. Noach, D. Cahen en dr. H.J. Voerman, dank ik voor de goede sfeer en veelal prettige omstandigheden waaronder ik mijn dagelijkse bezigheden kan uitoefenen. Beste Cees, Cees, Leslie, Djuna en Bert, ik ben er trots op deel uit te maken van een groep internisten die zich volgens de Elsvier 2007 'de beste van Nederland' mag noemen. De unieke combinatie van bloedserieuze patiëntenzorg en een grote dosis humor is een omgeving waarin ik met veel plezier functioneer. Hoewel geen deel meer van de maatschap wil ik dr. ir. J.J.M. van der Hoeven hier noemen. Beste Koos, het was een genoegen om tot je vertrek naar Alkmaar met je samen te werken. Ik heb veel van je geleerd, vooral op het voor mij grotendeels onbekende terrein van de geneeskundige oncologie. Je inzet en persoonlijke zorg voor je patiënten (en collega's), die nooit tevergeefs een beroep op je zullen doen, is mij nog steeds tot voorbeeld.

De secretaresses van onze maatschap, Marga Claasen, Carla Bakker, Henny van der Vis, Simone Walstra, Anja Heineman, Tonny van Weerdenburg en Esther Kunnen, dragen in hoge mate bij aan de prettige werkomgeving in Ziekenhuis Amstelland. Ik wil hen daarvoor danken. Mijn speciale dank gaat daarbij uit naar Kitty van der Sluis, beste Kitty, door de jaren heen ben je steun en toeverlaat geworden niet alleen voor mij, maar vooral ook voor de patiënten in 'onze' praktijk. Je overzichtelijke manier van werken en je nauwkeurigheid helpen mij om het hoofd te kunnen bieden aan het 'verschrikkelijke postkantoor' dat de dagelijkse praktijkvoering met zich meebrengt. Dit heeft mij de ruimte gegeven, die nodig was om dit proefschrift te kunnen afronden.

Ik dank Martin van Putten voor zijn hulp bij de voorbereiding op de verdediging en voor hele dikke sigaren.

Ik dank alle patiënten, die in een moeilijke periode van hun leven bereid waren om deel te nemen aan de studies in dit proefschrift.

Ik dank mijn beide paranimfen, Cees Rustemeijer en Jesse van Akkeren, voor hun bereidheid mij tijdens het reeds lang gevreesde uur ter zijde te staan. Cees, ik hoop dat jouw rustige overwogenheid, die ik bewonder, een klein beetje op mij zal afstralen die dag. Jesse, goede vriend, medeontwerper van het 'stegosaurus model' voor de IC patiënt, ik ben blij dat je me

wilt ondersteunen tijdens de ceremonie, hoewel ik liever een rondje met je rijd op de mountainbike door de Schoorlse duinen.

Ik dank mijn ouders, Jan en Tineke Timmers, voor hun liefde en steun en voor het feit dat zij het waren die mij stimuleerden en de kans boden om te gaan studeren en arts te worden.

Lieve Jozien en Annemarieke, ik ben blij dat ik hier niet hoef te schrijven dat ik nu weer tijd krijg om naar jullie hockeywedstrijden te komen kijken. Ik heb er niet veel gemist en blijf jullie trouwste supporter. Ik ben trots op jullie! Jootje, dank voor je hulp bij de voorkant van dit proefschrift.
Lieve Erica, dat wij elkaar begrijpen ook zonder woorden en voelen wat de ander voelt zelfs zonder elkaar aan te kijken, is mij zo veel waard. Dank voor je liefde, vertrouwen en vooral geduld met mij. Het kortstondig geluk dat het voltooien van dit proefschrift met zich meebrengt weegt niet op tegen de Landerumer heide in de avondzon in jouw gezelschap.
Tenslotte, maar niet op de laatste plaats dank ik God, die aan het begin en einde is van alle dingen.

Curiculum vitae

Gert Jan Timmers werd in 1966 in Leiden geboren. Na het behalen van het VWO diploma aan het Chr. Lyceum Dr. W.A. Visser 't Hooft te Leiden, begon hij in 1984 met de studie geneeskunde aan de Vrije Universiteit te Amsterdam. Het artsexamen werd in 1992 (cum laude) afgelegd. Vanaf 1993 specialiseerde hij zich in de interne geneeskunde, aanvankelijk in het Andreas Ziekenhuis te Amsterdam (opleiders dr E.H. Nauta, later dr. E. Monasch) en vervolgens in het VU Medisch Centrum te Amsterdam (opleider prof. dr. J. van der Meer). Het laaste jaar van de opleiding werd al besteed aan werkzaamheden binnen het aandachtsgebied hematologie (hoofd van de afdeling prof. dr. P.C. Huijgens). In 1999 werd hij geregistreerd als internist en in 2000 als hematoloog. In datzelfde jaar werd begonnen met het onderzoek zoals beschreven in dit proefschrift en volgde toetreding tot de maatschap internisten in Ziekenhuis Amstelland (destijds Ziekenhuis Amstelveen). Binnen dat ziekenhuis is hij momenteel werkzaam als algemeen internist met als aandachtsgebied de hematologie en oncologie. De afgelopen jaren vervulde hij binnen het ziekenhuis verschillende bestuurlijke functies. Momenteel is hij lid van het bestuur van de medische staf, is hij ondermeer betrokken bij de vormgeving van het samenwerkingsverband tussen huisartsen en het ziekenhuis in het kader van een nieuw op te zetten Eerste Hulp afdeling en bij de implementatie van een electronisch patientendossier (EPD) in Ziekenhuis Amstelland.